EXPERIMENTAL EVIDENCE FOR A PHYSIOLOGICAL MODEL OF MEMORY AND LEARNING

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SUMMARY

PART I

The experimental evidence for multiphase memory is presented and several physiological models are discussed which taken together can account for a tremendous body of experimental data concerning memory and learning. The experimental results discussed fall primarily into three categories: electroconvulsive shock data, the effects of drugs, and the so-called memory transfer experiments. Together these experiments suggest that memory consists of at least two distinct, though possibly not independent, phases. One phase, known as short-term memory, consists of a reverberatory electrical activity which is set up in interconnected self-reexciting neuronal circuits and does not require the synthesis of new macromolecules. The other, long-term memory, consists of structural modification of some of the synapses involved in the reverberatory activity. These modifications result in permanent facilitation for the passage of electrical signals in the particular network involved and require the synthesis of new protein molecules.

A derepressor hypothesis is presented which can account for the coupling of the short-term electrical activity and the synthesis of the protein associated with the long-term effect. Additionally, a general way in which these proteins could be involved in establishing the neuronal networks which constitute the physiological basis of long-term memory, as well as a way in which they could be involved in the memory transfer phenomenon, is suggested.

PART 2

Mice of the C57BL/6J strain, which are normally nonsusceptible to audiogenic seizure, can be made susceptible by prior intense auditory stimulation. Reasons for believing that the neural mechanisms responsible for the effect of acoustical priming may be similar to those discussed in Part 1 are given and experiments are described which attempt to transfer the effect of acoustical priming in a manner similar to the transfer experiments discussed in Part I involving more conventional learned behavioral patterns. The suppositions forming the basis for these experiments were: (1) Acoustical priming results in neural mechanisms which are in some manner similar to those initiated by a learning situation. (2) The structural changes within the brain which cause the animal to respond differently when the acoustical stimulus is presented at a later time are mediated by macromolecules (probably polypeptides). (3) The injection of these macromolecules into naive animals will alter their brain structure in a manner similar to the changes which they normally would have caused in the donor animals' brains and thereby affect the recipient animals' responses to acoustical stimuli.

The hypothesis based on these suppositions was that injection of brain extract from acoustically primed mice can increase audiogenic seizure susceptibility in non susceptible strains of mice.

Naive mice were injected with extract taken from the brains of primed mice. The experiments used litter-mate controls, which consisted of both non-injected mice and mice injected with either saline or extract from non-primed donors. It is concluded that injection of brain extract

from acoustically primed mice increases susceptibility to audiogenic seizure in C57BL/6J mice.

In a separate experiment it is shown that body weight is an important paramater affecting the acoustical priming results. At 21 days of age acoustical priming is very effective for C57BL/6J mice weighing less than 7.5 gm whereas it is much less so for C57BL/6J mice weighing more than 7.5 gm.

PART 1

PHYSIOLOGY OF MEMORY AND LEARNING

CHAPTER I

INTRODUCTION

One of the most fascinating and complex problems which science faces today is the question of how mind arises from brain. Just what are the physiological events which correspond to thinking, feeling, learning, etc.? The answer to these questions will be, without a doubt, one of the most significant scientific accomplishments in the history of mankind. As a result of the developments of the past decade or so, we now have a reasonable hope that this answer is within our grasp.

In the context of this dissertation the words memory and learning are used as defined by Richter (1966). Richter gives the biological meaning of learning as "a general term for the reorganization of behavior as a result of individual experience" and the meaning of memory as "the capacity of an organism to behave in a way modified by previous experience."

A promising model for memory is that memory consists of at least two distinct, though possibly not independent, phases. One phase known as short-term memory is temporary and liable; the other, long-term memory, may be permanent and is very stable. A working hypothesis is the following:

1. The nervous system is "prewired" by the developmental process. That is, the synaptic connections between neurons are a result of development rather than learning and, as long as they remain nonfunctional, do not represent memory.

2. As a result of stimulus input, electrical activity is initiated in tridimensional neuronal networks. If the stimulus is sufficiently strong, a reverberatory activity is set up in interconnected self-reexciting neuronal circuits such that the electrical signals pass through the networks many thousands of times. This represents a temporary memory phase and does not require the synthesis of new macromolecules.

3. If the stimulus is sufficiently strong, synaptic transmission at some synapses become permanently facilitated or perhaps inhibited for the passage of electrical signals in the particular tridimensional network. These changes require the synthesis of new protein molecules which presumably modify the chemical and physical structure of the synapses. Such modifications represent the long-term phase of memory, the formation of which is referred to as consolidation.

In support of point two of the working hypothesis is the fact that reverberating signals can be demonstrated for as long as an hour, back and forth between the cerebral cortex and the thalamus after a strong sensory signal barrages the brain. Furthermore, these reverberating signals are localized to certain areas of the cerebral cortex depending on the type of sensation that is experienced (Guyton 1969 page 343). Using multiple microelectrodes in the thalamus of cats, Verzeano and Negishi (1960) found transient reverberation of neural activity resulting from stimulation. Oniani (1971) concludes a review of some of his studies of alimentary responses based on short-term memory by stating that his results could best be understood by supposing "that one of the main determining factors in the organization of neurophysiological basis of recent memory

is the reverberation and post-tetanic potentiation in the functional nervous circuits of the brain. In particular, reverberation of impulses and facilitation of the conduction in the neural networks of the limbic system seem to have an important role in the regulation of delayed alimentary conditioned reaction."

Although it now seems likely that reverberatory neural activity is the basis for short-term memory, it is clear that long-term memory cannot be attributed to enduring reverberation. Long-term memories have been shown to survive treatment which severely disrupts electrical neural activity (e.g., electroconvulsive shock, spreading depression, cathodal polarization). The fact that some memories last a lifetime and are extremely stable requires that changes occur in the matter of which the brain is constructed, and therefore formation of long-term memory almost certainly involves protein synthesis.

Figure 1 (John 1971) is a schematic representation which includes a third phase of memory.

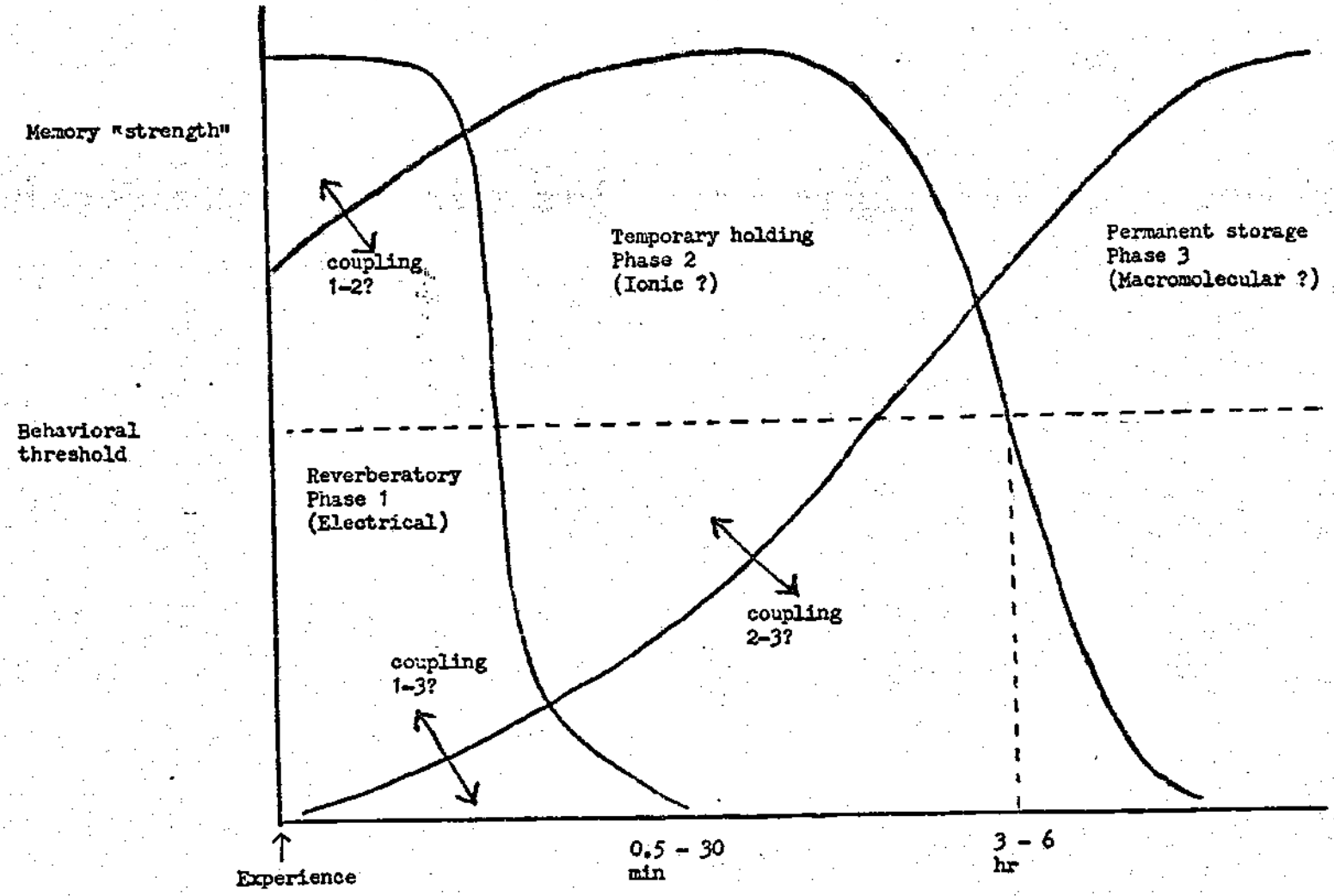

Figure 1. A Schematic Representation of the Stages in Memory.

CHAPTER II

EXPERIMENTAL EVIDENCE FOR MULTIPHASE MEMORY

Electroconvulsive Shock

Most of the evidence for a short-term memory phase comes from electroconvulsive shock (ECS) data in humans and laboratory animals. These results indicate that ECS can cause permanent retrograde anmesia (RA) and that there is a temporal gradient associated with the degree of amnesia which seems to be a function of the material to be remembered, the severity of the ECS, and the species involved. It is believed that ECS interferes with the mechanisms by which long-term memory is established.

In order for ECS results to be considered as evidence for a short-term memory phase, the disruptive effects of ECS must be permanent in at least some cases. Many researchers have demonstrated amnesia resulting from ECS for periods from one month to six weeks after treatment (Chevalier 1965; Luttges and McGaugh 1967; Geller and Jarvik 1970).

The existence of a temporal gradient for ECS data is a generally accepted fact. That is, almost all investigators have found that the measure of retrograde amnesia is greater the shorter the training-ECS interval and that beyond a certain interval, ECS is no longer effective in producing RA. The temporal gradient has obvious theoretical importance in that it must reflect some changing process within the organism and may be interpreted to mean that the ECS disrupts a consolidation process and that the interval between training and ECS treatment is a relative measure of the amount of consolidation which has already taken place. How-

ever, a factor which greatly complicates the theoretical interpretation of this data is the wide discrepancies in the published intervals after training for which ECS can produce demonstrable amnesia (Chorover and Schiller 1965; Pinel 1969; Kopp et al. 1966; Robustelli et al. 1969; Herz 1967).

Drugs

In contrast to short-term memory, long-term memory has been studied not with ECS but by administration of drugs, particularly drugs which inhibit the ability of the organism to synthesize protein. In a variety of animal species and a number of behavioral tasks, inhibition of protein synthesis before or shortly after learning, appears to interfere with retention of the learned response. This has been interpreted by most investigators to be due to an interruption of events which would normally lead to long-term memory storage.

Barondes (1970) injected mice with acetoxycycloheximide (AXM) and, when protein synthesis was inhibited by 95% or more, trained them to escape shock in a T-maze. The AXM injected mice learned in the same number of trials as a saline injected control group, and when tested three hours later both groups showed normal memory. These results indicate that the ability to synthesize protein is not necessary for either learning or short-term memory. However, when tested from six hours to seven days after training, retention was markedly impaired in the AXM injected group. Barondes also found that AXM injections from five hours to five minutes before training produced performance deficits in mice when tested seven days later. Injections immediately before, immediately after and five

minutes after training were also effective but less so, and post-training injection at 30 minutes and 24 hours produced results which were not distinguishable from controls.

With AXM injection protein synthesis does not return to normal until four days after injection. However, with cycloheximide, which is just as effective in inhibiting protein synthesis, the capacity to synthesize protein has largely returned to normal three hours after injection. With cycloheximide injection long-term memory was impaired even though protein synthesis was possible at a time when the animal showed normal retention of the learned task (Barondes and Cohen 1968). This indicates that persistence of the cognitive information acquired from training and an intact cerebral protein synthesizing capacity alone are not sufficient for formation of long-term memory. Barondes and Cohen further found that by inducing a state of "arousal" by foot-shock or amphetamines three hours after training, they could induce the formation of long-term memory. However, this effect could be blocked by the resumption of inhibition of cerebral protein synthesis.

Inhibition of protein synthesis has also been shown to block formation of long-term memory in goldfish (Agranoff 1970a).

Both ECS and inhibition of protein synthesis can prevent formation of long-term memory. They are also similar in that neither has an immediate effect on the performance of the learned behavior; that is, some temporary memory phase survives and allows the animal to continue performing for several hours before the anmesic effect is apparent. (Geller and Jarvik 1968; McGaugh and Landfield 1970; Barondes 1970). This suggests the

possibility of some relationship between their modes of action. Agranoff (1970a) notes that ECS is accompanied by a transient decrease in protein labeling, but that the magnitude of the decrease is less than observed with doses of AXM, which produce far less memory loss. He concludes that ECS blocks memory by some means other than inhibition of protein systhesis.

Actinomycin-D, a drug which inhibits DNA dependent RNA synthesis has also been shown to prevent formation of long-term memory while not affecting learning or short-term memory (Agranoff et al. 1967; Cohen 1970; Codish 1971).

In addition to drugs which inhibit the formation of long-term memory, there are also drugs which facilitate its formation. Lashley (1917) reported a facilitation of maze learning in rats with low doses of strychnine sulfate, and these results have been confirmed by McGaugh and Petrinovich (1965). McGaugh (1966) used post-training strychnine injections and tested the rats after the effects of the drug had worn off to demonstrate facilitation of learning in a variety of tasks. He concluded that central nervous system stimulants can facilitate learning by enhancing memory consolidation. In addition to strychnine, post-training injections of picrotoxin, Metrazol, caffeine, and amphetamine have been shown to be effective. McGaugh (1967) found that injection of strychnine up to three hours after ECS almost eliminates the disruption of consolidation of one-trial learning. This is similar to the arousal effect found by Barondes and Cohen (1968) with cycloheximide mentioned earlier.

Pearlman et al. (1961) found that mice exposed to a one-trial learning situation showed no retention of the experience if they received

ECS ten minutes later. However, animals which received an injection of strychnine immediately after training are uneffected by ECS ten minutes later. Their experiment indicates that the facilitory effect of the central nervous system stimulants is the result of an increased rate of consolidation rather than a prolonging of the consolidation process. McGaugh and his co-workers have further strengthened this position. McGaugh et al. (1962) found that the Tryon maze-bright rats were superior in maze learning to maze-dull rats only in massed trials. If the trials were spaced five minutes or more apart, the two groups of animals were not significantly different. The experimenters concluded that the strains differed in the rate of consolidation and that the time between trials allowed the slower consolidating strain to "catch up" with the maze-bright strain so that they started each trial on an equal basis. McGaugh et al. (1961) found that injections of the central nervous system stimulant 5-7 diphenyl-1-1-3 diazadamantan-6-01 into both strains eliminated the difference between them; the drug has the same effect as separating the trials, and therefore apparently increases the rate of consolidation in the maze-dull strain.

Intermediate Holding Phase Of Memory

John (1971) presents arguments for including a third phase of memory, a temporary holding phase, possibly due to unusual molecular or ionic concentrations. Agranoff (1970a) found that, while either AXM or puromycin administered immediately after training could effectively block formation of long-term memory in goldfish, performance was not immediately effected but rather decayed gradually over a three day period. Treatments

in mice such as AXM injection or ECS, which will ultimately block formation of long-term memory, have been shown not to effect performance until several hours after training (Barondes 1970; Albert 1966a, 1966b; McGaugh and Landfield 1970).

John feels that the intermediate trace is of too long duration to be plausibly attributed to reverberation and that its ability to survive ECS, spreading depression, and cathodal polarization further excludes this explanation.

It has been suggested that the development of the temporary holding phase may be time dependent, as the development of the long-term phase seems to be. McGaugh and Landfield (1970) found that if the ECS was given eight seconds rather than 20 seconds after training there was not a gradual decrease in performance, but instead, performance was consistently poor. In light of this result, the curve representing the temporary holding phase in John's schematic representation of the stages of memory should start below the behavioral threshold, as it does in the representation of Halstead and Rucker (1970).

Memory Transfer

Some of the most convincing evidence for chemical involvement in at least part of the memory process has come from the success of the so-called memory transfer experiments. In general, donor animals are trained to respond to a particular stimulus in a particular way. A chemical extract is prepared from the donor animals brains and introduced into untrained recipients, which are then tested for the response for which the donors had been trained.

The memory transfer experiments have stirred considerable controversy. In 1966 a "failure to replicate" article was published in Science and signed by 23 authors (Byrne et al. 1966). This article cast serious doubt on the initial memory transfer results. However, since that time the original results have been replicated many times over and considerably extended in at least 33 laboratories all over the world (Ungar 1972). The phenomenon itself can no longer be questioned, but doubt still remains as to its significance. Many authors maintain that the behavioral modifications result from some generalized chemical effect, similar perhaps to strychnine injections, rather than from involvement of the injected molecules in a specific way in the particular memory trace. The specificity of the recipient animal's response is now too well documented, however, for generalized effects to represent a reasonable explanation of the results (Jacobson et al. 1965, 1966; Ungar 1967, 1969; Essman and Lehrer 1967).

Although stimulus and response specificity appears to have been demonstrated, it has still not been clearly shown that the transfer effect is dependent upon and specific to the learning of the donors (Dyal 1971).

Many of the original researchers worked under the assumption that mRNA was the molecule which was responsible for the transfer effect. It is now reasonably certain that the behaviorally active substance is a polypeptide (Rosenblatt 1970), and that the results obtained with RNA extracts were actually mediated by peptide impurities for which RNA acted as a carrier (Ungar and Fjerdingstad 1971; Guttman 1972).

There have been many experiments to determine the circumstances

under which memory transfer can be successfully demonstrated (Reinis and Mobbs 1970; Ungar 1970b; Adam and Faistz 1967). It appears that the memory transfer phenomenon involves only recently learned behavior that is based on the recipients' innate responses. This is perhaps disappointing to many whose attention was concentrated on the recipient and who anticipated spectacular applications involving the imparting of knowledge in chemical form. These people have missed the point of the phenomenon. As Ungar (1970a) points out, "the outstanding feature of the experiments is that they enable us to detect the chemical correlates of learned information in the brain of the donor and provide us with a technique for assaying the material and assist us in its isolation and characterization."

In perhaps the most significant developments since the initial memory transfer results, Ungar and his co-workers have been able to 1) isolate and identify the amino acid sequence of a particular behavior-inducing substance (Desiderio et al. 1971), 2) synthesize the substance and show in rodents that the behavior-inducing effect of the synthetic substance is not distinguishable from that of the peptide extracted from the brains of donor rodents (Parr and Holzer 1971), and 3) show that the synthetic substance has the same behavior-inducing effect when injected into goldfish (Guttman et al. 1972). The substance discussed above elicits a dark-avoidance response and has been named scotophobin by Ungar. In addition to scotophobin, five other behavior-inducing substances have been partially purified and characterized, each inducing one behavioral pattern out of the several for which they were tested (Ungar 1972).

CHAPTER III

MEMORY MECHANISMS

John (1971) gives two possibilities for the mechanism of memory. The first, which is the one he favors, is a probabilistic model associated with the ideas of the "equipotentiality" and "mass action" and called the mode model.

> The configuration of activity in the cells of the network at any time defines the state of the network. The temporal sequence of states in the network defines a mode of activity. Modes of activity involving greater coherence than is characteristic of ongoing background activity in the individual cells of the network constitute information.... The temporal and spatial configuration of stimuli impinging upon the network during an experience activates a particular mode of response. Increase in the probability of coherent activity in that mode constitutes the stored memory. (John 1967 pages 64-65).

Different items of information are represented as different patterns of combination or sequences of activity of different cells.

The second possibility John lists is the deterministic cellular-connection model. Memory is stored in diffuse or multilocalized anatomical systems involving cells in many brain regions. These cells are connected by synapses which were laid down by the developmental process but whose functional properties have changed as a result of coordinated neuronal activity associated with learning. Activation of specific memory requires the discharge of particular cells in the system.

These neuronal networks are initially established as a result of the spatio-temporal pattern of receptor activity which fires specific excitatory and inhibitory circuits in such a way that a particular re-

verberatory network is established. This second model is the more intuitively reasonable one and seems more consistent with the memory transfer results. To explain memory transfer with the mode hypothesis would require that the molecules in the extract modify neurons over a very large area of the brain. The following discussion will be in terms of the cellular-connection model.

From the results obtained in the inhibition of protein synthesis experiments, it seems reasonably certain that the ability to synthesize protein during some critical period associated with the learning experience is essential for the formation of long-term memory. Furthermore, the memory transfer results indicate that polypeptides are involved in a specific way in the formation of this long-term memory. Although a step by step explanation of the memory mechanism is not possible at this time, a general, intuitively reasonable model can be developed which is consistent with most of the experimental results. John (1967, 1971) has proposed the derepressor hypothesis to account for production of the specific polypeptide, Ungar (1968), and others have proposed mechanisms by which the polypeptides express themselves as long-term memory, and recent results from immunogenetics may shed light on genetic involvement in memory.

Derepressor Hypothesis

Development from a single fertilized egg insures that each cell contains an essentially complete genome. However, the genetic potentials present are for the most part repressed. The capacity for synthesis is determined and constrained by the DNA structure, but the momen-

tary expression of these potentialities is in many cases regulated by cellular activity. Watson (1970 pages 440-442) discusses the mechanisms by which genetic expression is controlled in bacteria. Regulatory genes are responsible for production of a special group of protein molecules called repressors. Under certain circumstances these repressor molecules are able to bind to the DNA in such a way as to prevent transcription of mRNA corresponding to a particular gene or group of related genes. Repressors do not permanently prevent specific mRNA synthesis; all repressor molecules can exist in both an inactive and an active form depending in general on whether they are combined with their appropriate inducer (derepressor) or corepressor. The attachment of an inducer inactivates the repressor whereas the attachment of a corepressor changes it into an active form. Inducers and corepressors are collectively referred to as effectors. The effectors are bound to the repressor by weak bonds which are rapidly made and broken, allowing the repressor state to adjust quickly to physiological needs. That is, the cytoplasmic concentration of specific metabolites controls the activities of the repressors. (See Figure 2, which is taken from Watson (1970) page 445). The specific nucleotide sequence which binds the active repressor is called an operator and the group of genes controlled by the repressor is called operon. Operons may code for several different, though usually related, proteins, but they code for a single mRNA molecule.

The increased RNA synthesis and the nucleotide base ratio change associated with learning (Hyden and Egyhazi 1962, 1963, 1964; Hyden and Lange 1965) suggest derepression. Many authors have outlined mechanisms

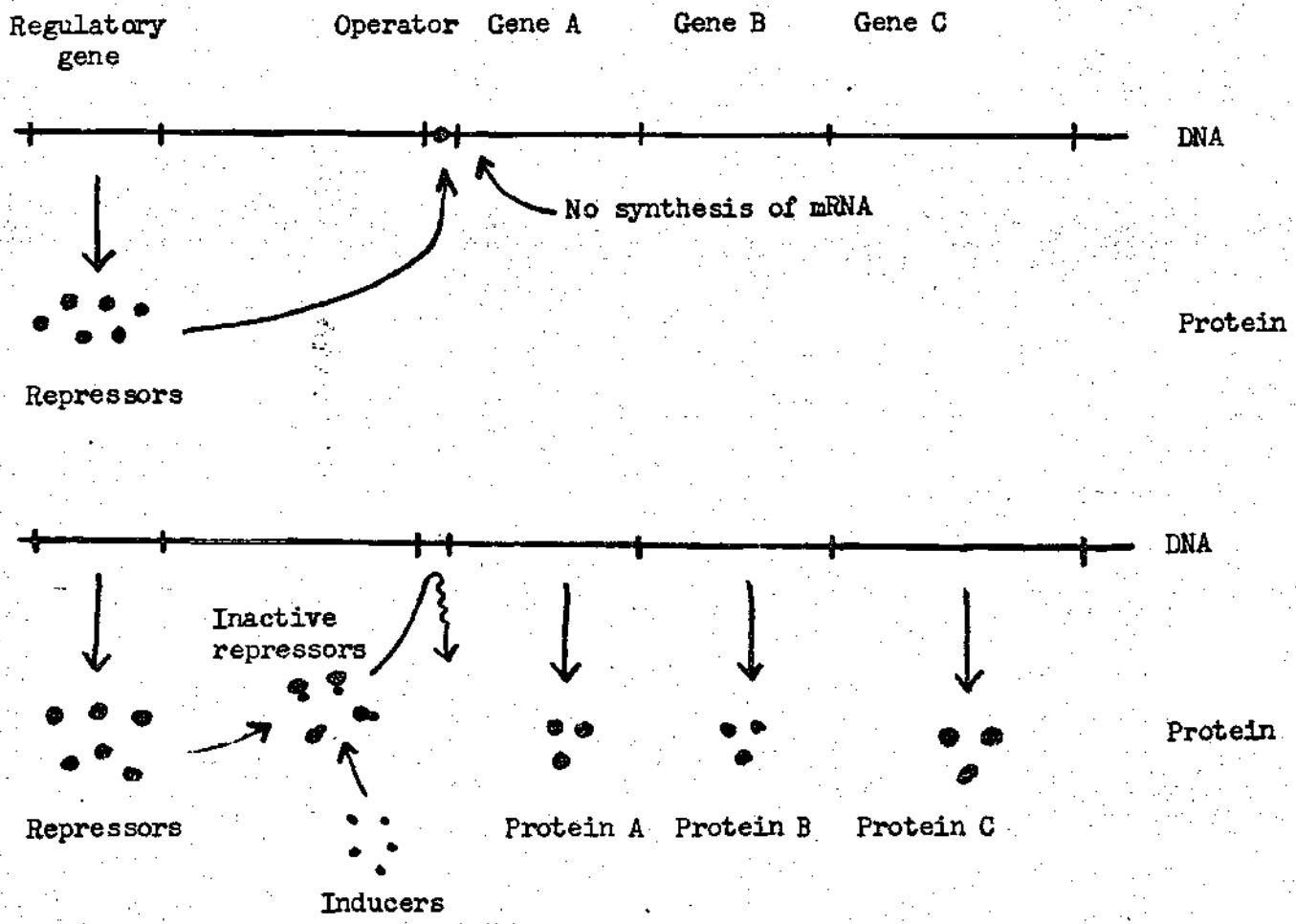

Figure 2. A Schematic Representation of the Means by Which the Interaction of Repressor, Inducer, and Operator Controls the Synthesis of Protein.

which involve derepression in the chain of events leading to long-term memory formation (Bonner 1966; Flexner 1968). Perhaps the most detailed description is that due to John (1967 page 131 and following, 1971), in which he outlines the derepressor hypothesis:

1. In any cell much of the potential for synthesis of specific substances inherent in the DNA structure is repressed.

2. Sustained participation of a neuron in representational activity causes a shift in the concentration of cytoplasmic materials, resulting in the derepression of an inhibited synthesis.

3. The resulting alteration in cytoplasmic constituents has two consequences: a) Derepression of that synthesis is thereafter sustained. b) The reactivity of the neuron to patterns of stimulation is altered.

Under normal circumstances cytoplasmic concentrations are maintained at approximately steady state levels. Firing of the neuron causes a change in the permeability of the membrane and therefore a change in cytoplasmic concentrations, particularly of the ion populations. While the change in concentration due to a single discharge would probably dissipate, it is possible that sustained activity resulting from participation in a reverberatory neuronal network could produce a critical shift in effector concentration. That is, the effector concentration could reach a level sufficient to affect derepression of a particular operon. If each cellular discharge is considered to contribute a unitary increment in concentration of the effector then there is some minimum rate-duration combination for reverberation to produce a critical shift in effector concentration. Interruption of reverberation before this time

(ECS, spreading depression, cathodal polarization for example) would result in failure to achieve the critical shift and residual concentrations (intermediate holding phase?) would be expected to dissipate gradually because of metabolism and diffusion. Eventually normal concentration levels are restored and there is no lasting effect of the experience in that neuron; no long-term memory has been formed.

If more complex learning situations require larger reverberatory loops then according to the derepressor hypothesis the consolidation time would be correspondingly longer, perhaps longer than reverberation could be sustained. This would require repeated training to cause a lasting behavioral effect.

As discussed earlier some animals given ECS treatment do not develop long-term memory but do show normal retention several hours after treatment. McGaugh (1967) has found that "arousal" such as strychnine injection given three hours after treatment can eliminate the RA effect of the ECS. What conceivably is happening here is that the ECS disrupts reverberation before a critical shift in effector concentration occurs. The abnormal concentrations however, "mark" the pathways and mediate behavior until those concentrations have returned to normal, after which there is no memory of the training. The effect of arousal then would be to reinitiate reverberation which would then affect the critical shift and long-term memory would be formed.

Similar results obtained by Barondes and Cohen (1968) using cycloheximide rather than ECS to block formation of long-term memory, can also be explained in this same way. Recall that mice given cycloheximide

injections prior to training did not form long-term memory even though protein synthesis had returned to normal three hours after training at a time when the mice showed normal retention of the learned task. However, if a state of "arousal" was induced at this time by a foot-shock reminder or amphetamine injection, long-term memory did develop. If by the time protein synthesizing ability had returned the critical shift in effector concentration had dissipated, reinitiation of reverberation would be required for derepression and the subsequent consolidation of long-term memory.

It is interesting to note that in the strychnine injection experiments, the strychnine is effective in eliminating RA if the training-ECS interval is 20 seconds but not if it is eight seconds (McGaugh 1969). Recall that McGaugh and Landfield (1970) found that performance was high one hour after treatment with a 20 second training-ECS interval but not with an eight second interval, though both eventually produced RA. This was given as evidence for the temporary holding phase suggested by John (see Chapter II). This is consistent with identifying a sub-critical shift in effector concentration with the temporary holding phase of memory. That is, reverberation for eight seconds is not sufficiently long, in this case, for concentration changes to achieve the behavioral threshold, and additionally allow "arousal" to reinitiate reverberation.

Lubin (1963, 1964) indicates that the rate of protein synthesis in various bacterial cells was selectively affected by decreases in intracellular potassium ion concentration and Lubin and Ennis (1963) found that the effect of the ammonium ion was even more marked than that of

potassium. "The evidence that protein synthesis is responsive to electrolyte concentration lends some plausibility to the assumption that the ionic shift resulting from a sustained pattern of neural activity may act as an effector or that the effects of ionic shift on enzyme activity of protein synthesis may play this role " (John 1967 page 143).

We have seen a possible mechanism whereby sustained, coordinated neural activity results in the production of a previously repressed polypeptide. In order to be consistent with the suggested memory mechanism, this polypeptide must alter the response characteristics of the neuron in such a way as to provide for retrieval of the information which is represented by the particular reverberatory neural activity. For instance, if a mouse steps off a platform and is shocked, then the stepping off the platform-pain information is represented by electrical activity involving a number of neurons in the brain. As a result of memory this particular electrical activity, or activity similar in some degree to it, can be initiated by situations other than stepping off the platform and being shocked. The stepping off the platform-pain signals could be initiated simply by the presence of the mouse on the platform. That is, the coordinated activity of a certain set of neurons results in the coordinated activity of another set of neurons which initially would not have been involved unless the mouse had actually been shocked. The ability of the one set of neurons to excite the other set of neurons results from modification of the synapses connecting these sets as a result of learning. That is, prewired, previously nonfunctional synapses are converted into functional synapses. It is reasonable to assume that the synaptic modi-

fication is affected by the derepressed polypeptide which is carried to the synapse by axoplasmic flow (Ochs 1966). How the polypeptide actually converts the synapse from a "naive" to a "trained" snyapse has not been discovered yet but many feel that it involves the polypeptides becoming a part of the synaptic membrane (Byrne 1970; Ungar 1968a; Halstead and Rucker 1970). Others stress changes in the neurotransmitter concentration as the mechanism regulating intercellular communication (Arganoff 1970b). It is important to note that memory does not reside in the molecules themselves but rather in the synaptic connections which they effect.

Another possibility is that the synpatic modifications consist of a greater physical contact between the pre- and post-synaptic membranes, perhaps, a decrease in the synaptic gap (Barondes 1965). An interesting feature of this concept, as Barondes points out, is the known importance of intercellular contact in regulating cellular activity (Watson 1970 page 591). In this way, the synaptic modification itself could result in biochemical changes, the effect of which would be continued derepression of the particular polypeptide. Small interlemnal elements which extend across the synaptic cleft (Schade and Ford, 1965 page 92; McGreer 1971) represent logical structures for synaptic modification.

Most authors who discuss derepression of the polypeptide as a critical step in memory formation, assign a role of effector mimic to the polypeptide in a manner similar to John's derepressor hypothesis. The rationale for this is the observed turnover rates for cellular protein. It is tempting to consider mechanisms which would make continued synthesis of the polypeptide unnecessary once consolidation had taken place.

Perhaps if the polypeptide were to assemble into the synaptic membrane it is possible that it could be protected from the metabolic activity in the cytoplasm. Booth (1967) states that "it may be possible for membrane properties to be radically and permanently altered by the local formation of...macromolecular complexes of such conformation as to virtually inaccessible to degradative enzymes." As an example he gives the formation of a protein globule by an antigen reacting with an antibody such that the globulule has a hydrophobic exterior. A feature which could be explained by binding of the critical polypeptide to the membrane is the inability to transfer instinctive behavior or behavior learned long before the extraction.

Unfortunately there is some evidence that this is not the case. Reinis (1968) was able to block memory transfer by combining intraperitoneal injection of brain extract from trained animals with intracranial injection of antinomycin-D. Since actinomycin-D selectively blocks RNA transcription on DNA templates, Reinis feels that the change in the behavior of the recipient animal is probably caused by the derepressor effect of some material present in the extract. Reinis and Mobbs (1970) explained the inability to transfer instinctive behavior or behavior learned long before the extraction by assuming that the highest quantity of active factor is present during training and decreases once the task is well learned. It is known that "great variation can exist between the amount of a protein present when it is needed and when the environmental conditions are such that it would serve no useful function" (Watson 1970 page 438). Presumably if the concentration of active factor was extremely low, no transfer would occur.

Specificity of the Derepressed Polypeptide

At this point we have a general theory which can be used to explain the essential features of both the ECS and the drug effects on memory. Stimulation produces activity in a particular set of neurons. Under certain circumstances this stimulation will result in a reverberating signal which is the physical basis for a temporary phase of memory, and which does not require the synthesis of macromolecules. If reverberation is allowed to continue, a critical shift will be produced in an effector molecule which will result in the production of a previously repressed polypeptide. This polypeptide will alter the response characteristics of the neuron in such a way that certain neuronal networks can be excited or inhibited under circumstances other than those required for the initial excitation or inhibition. This is the physical basis for a long-term phase of memory.

However, if this general theory is to explain the memory transfer results we must further assume not only that the polypeptide is somehow specific to the learning situation but that it can mimic consolidation when introduced into naive animals. Obviously, this greatly complicates what previously had been a conceptually simple mechanism. Without the memory transfer results we could have assumed that there was only one species of polypeptide responsible for the synaptic modifications and that specific memories were the result of which synapses were affected. Although we can still assume that the basis of memory is specific neuronal networks, we must now require that the synaptic modifications which form these networks result from highly specific polypeptides.

Is there any reason to believe that specificity to this degree exists? Can the genome possibly code for that much information? In short, is there a plausible explanation for the memory transfer results? The answer is, yes!

It is a well known fact that cells from different organs of the body have specific affinities for each other. That is, if liver and kidney cells are mixed, the two types will form separate aggrates. It is now known that this affinity results from specific substances which coat the different cells and allow them to establish connections only with those cells which have the same chemical affinity. This process can go much further in such a highly differentiated assemblage of cells as the nervous system. Sperry (1963) by studying nerve regeneration, found that surgically scrambled fibers somehow unsorted themselves and managed to home in on their original and proper central nerve terminals. He concluded that "the cells and fibers of the brain and cord must carry some kind of individual identification tags, by which they are distinguished one from another almost, in many regions, to the level of the single neuron; and further, that the growning fibers are extremely particular when it comes to establishing synpatic connections, each axon linking only with certain neurons to which it becomes selectively attached by specific chemical affinities."

At first glance, it would seem as if the genome could not possibly represent enough distinct chemical labels to accomplish all the tagging suggested above. However, Roberts and Flexner (1966) were able to formulate a mechanism based 100% on known biochemical reactions which would

allow each neuron in the nervous system of a human being to be distinctively labeled and would involve only about 30 genes! A more convincing model for production of specific polypeptides by individual cells has been developed by Gally and Edelman (1970). Their recombination model is proposed as an explanation for antibody formation and will be discussed in more detail later.

Ungar (1968a) has proposed a mechanism which he feels is reasonable and can be used to explain the transfer results. He feels that there are certain innate patterns which correspond to behavior such as aversion to pain and food seeking when hungry, and that the initial phases of natural learning are based on these innate patterns. The simplest example of Ungar's mechanism is his explanation of classical Pavlovian conditioning. One of the innate patterns involved here is the one involving the unconditioned stimulus and the unconditioned response. For instance, when food is presented to a hungry dog, saliva starts to flow. The other innate pattern is the one corresponding to what will become the conditioned stimulus and its response. For instance, the dogs reaction to a bell. Under normal circmustances the probability of salivation being elicited by a sound stimulus is negligible. However, when the food and the bell are presented simultaneously, both patterns fire at the same time. Ungar suggests that when the neurons of the conditioned stimulus pathway, which have previously non-functional synapses with the neurons of the unconditioned stimulus, fire, they release a certain amount of their "specific connector substance." If the neurons of the unconditioned stimulus pathway fire at the same time or within a brief interval, they

can take up the substance released by the neurons of the conditioned stimulus pathway, and a temporary connection will be established between the two pathways. Repetition of conditioning will result in a more permanent connection, so that eventually there will be a functional pathway which relates the conditioned stimulus to the unconditioned response.

The polypeptide which encodes the connection will be a complex consisting of an amino acid sequence contribution from both pathways involved; a "composite word" (Ungar 1972). The permanent facilitation results when derepression is effected. Thus, elementary learning is based on establishing connections between innate patterns of response, which once established can serve in turn as a basis for the more elaborate forms of learning.

Ungar (1970a) suggests that this "identification system has developed as part of the evolutionary process of differentiation of the nervous system" which was discussed earlier with regard to Sperry's (1963) regeneration results. Szentagothai (1971) also feels that memory and learning may be an extension of the embryonic development of the nervous system. "It is then by no means inconceivable that memory and learning are but further steps of neural specification in the postnatal development."

Production of the "connector substances" could be accomplished as outlined by John's derepressor hypothesis, with the additional requirement that the derepressed polypeptides responsible for the connection are somehow specific to the two innate patterns involved.

It is clear that more complex learning situations, such as those involving a positive or a negative feedback of information, would require

a more elaborate relationship between the innate patterns involved. By utilizing inhititory as well as excitatory snyapses, Ungar is able to deal with these more complex learning situations in much the same way as with classical conditioning.

Ungar explains memory transfer in the following way: specific polypeptides responsible for establishing long-lasting connections between innate pathways in the trained animal can mimic this consolidation when introduced into the brain of naive animals. "When the connectors included in the extract injected into the recipient animal, fall in place in the neurons homologous to their site of origin, the nerve impulse follows the trail created by the training of the donors" (Ungar 1968a). These coded molecules do not actually transfer memory; certainly the recipient does not "remember" the training of the donor. What is transferred is a tendency to behave in a particular manner; an increase in the probability of a particular behavioral pattern. "To use the holographic metaphor, the behavior of the recipients is like a hologram that is based on a reduced number of grains and, therefore, is less percise than the complete picture" (Ungar 1970a).

The evidence for specificity of cellular connections has been discussed earlier and is unequivocal. Ungar's mechanism for memory transfer, however, goes beyond the ability of one cell to recognize another. He suggests that molecules produced in a certain very restricted region of the brain are able to "fall in place in the neurons homologous to their sites of origin" when injected into recipient animals. We must assume that the molecules responsible for the transfer actually accumulate in

the homologous regions as dilution throughout the body would give a small probability that the active factor would reach this region. There is some evidence that this is exactly what does happen.

Porter (as reported by Byrne 1970) has conducted experiments which indicate that homogenates of isotopically labeled liver or kidney, following intraperitioneal injection, would preferentially localize in the corresponding organ. Walter et al. (1956) has shown preferential localization of injected adult liver and heart homogenates into the homologous tissue of the embroyonic chicks. Stillwell el al. (1971) using intraperitoneal injection, demonstrated specificity for kidney, lung, olfactory lobe and brain (without the olfactory lobe) tissue. If a mechanism exists whereby molecules produced by heart tissue can upon injection, accumulate in heart tissue, then certainly one could exist whereby molecules produced in a restricted portion of the nervous system can do the same. Recall that Sperry (1963) has indicated that neurons can distinguish between other neurons just as heart cells can distinguish between cells from different types of tissue.

A certain amount of support is given Ungars's mechanism of memory transfer by results obtained by Mihailovic and Jankovic (1965). Their results as summarized by Booth (1967) are "that chemical specificity is sufficiently delimited anatomically and sufficiently similar from animal to animal to make it possible to inject a cat intraventricularly with some antibody to caudate nucleus tissue from another cat and to obtain an initial excitation and eventual loss of electrical activity localized to the caudate nucleus of the recipient."

If we accept the fact that the polypeptides responsible for synaptic modification associated with learning are in some way specific to that learning, we must then consider in what way they are specific. Is the mechanism instructional so that the structure of the representational molecule is somehow specified by the information to be stored (e.g., Fong 1968) or selectional so that one of a preexisting set of possible structures is allocated for a given representational function. This, of course, is exactly the question that was being asked until recently about antibodies. It is now known that the specificity of antibodies lies in distinct amino acid sequences, rather than conformational differences. This makes an instructional theory very unlikely and most researchers now favor the selectional theory of antibody formation (Burnet 1961; Gally and Edelman 1970; Jerne 1971). Ungar's work with scotophobin strongly suggests that a selective mechanism is also involved in the specificity of the polypeptides involved in memory consolidation.

The similarities between immunity and learning are striking. Both have lately been shown to involve specific amino acid sequences, a theory which was in both cases earlier rejected as requiring too many genes. Both involve long-lasting physiological changes resulting from a relatively brief "exposure" period. Also the most convincing models for both immunity and learning require that the individual cells involved, lymphocytes and neurons respectively, are capable of producing a limited number of highly specific polypeptides. For these reasons it is hoped that an explanation of the immune response will shed light on memory mechanisms.

An immunoglobulin consists of two different polypeptide chains

called heavy and light. Each chain can be divided into two regions, one called the variable region which is responsible for the antigentic specificity, the other called the constant region which is responsible for the effector functions of the antibody; that is, how the organism reacts to the antigen after it has been bound (Gally and Edelman 1970). The enormous range of antigen specificity results from a corresponding diversity of amino acid sequences in the variable portions of the antibody. Gally and Edelman (1970) have proposed a selectional mechanism to account for the diversity of the immunoglobulins. Their theory involves somatic recombination of V-genes (genes coding for the variable region of the antibody) followed by translocation to a C-gene (gene coding for the constant region of the antibody) to form a functional VC gene. This is represented in Figure 3 which was taken from Gally and Edelman (1970).

In this manner a large number of distinct polypeptide chains can be generated, the exact number depending on the number of V-genes and the average number of positions at which each V-gene differs from its neighbors.

In stressing sub-microscopic mechanisms as models to account for memory and learning it is important not to lose track of the fact that central nervous system functions are essentially macroscopic in nature. As discussed earlier, informational significance lies primarily in specific neural pathways. These neuronal networks are initially established partially as a result of the spatio-temporal pattern of the receptor activity which fires specific excitatory and inhibitory circuits. However, whether or not reverberatory activity results appears to be determined by

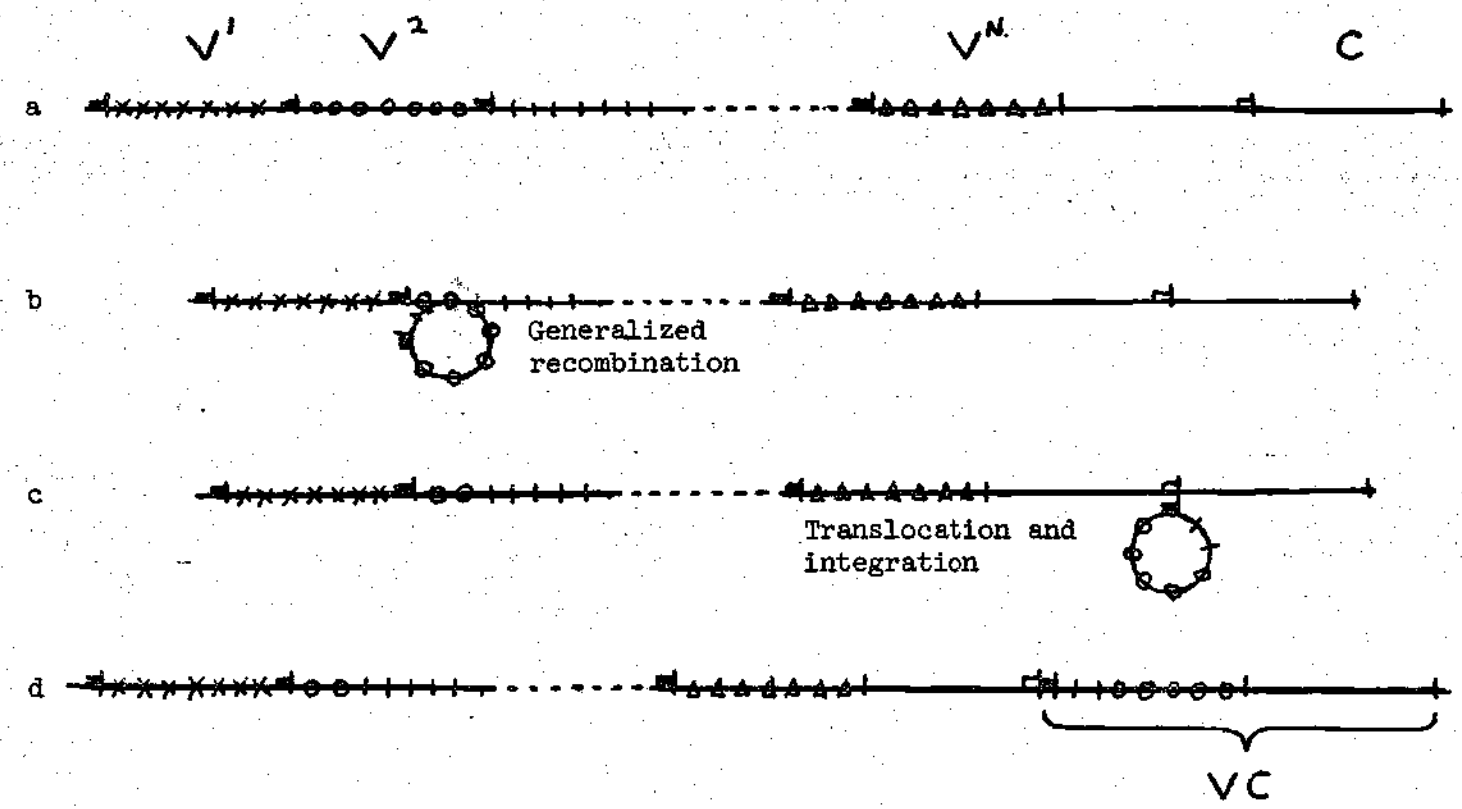

Figure 3: A Schematic Representation of a Recombination and Translocation Model for Antibody Specificity. a) Cluster of genes for polypeptide chain of antibody includes several tandemly arranged V-genes and a nearby C-gene on the same chromosome. b) Intrachromosomal generalized recombination to form an episome, which usually will contain base sequences occuring in two adjacent V-genes. c) The V-gene episome is reinserted adjacent to the C-gene. d) Integration of the episome results in a functional VC-gene.

an internal control center in the basal region of the brain (Guyton 1969 page 343; Torda 1970). Whether the particular information will be stored forever or almost immediately forgotten depends on the "state" of the mind and whether or not the information is capable of eliciting an emotional response. Specifically, the evidence indicates that the hippocampus is concerned with "the correlation of the sensory input with the motivational state of the organism" and with the consolidation of the appropriate emotional and behavioral response information into a permanent memory store (Smythies 1970 pages 147-148).

Therefore, under certain restricted circumstances, involving both external stimuli and internal control, reverberatory activity is established in specific tridimensional neural networks. An immeidate consequence of this reverberation is that transmission of electrical signals from neuron to neuron within the network is facilitated due to a shift in membrane potential (post-tetanic potentiation) and possibly an accumulation of transmitter substances at the postsynaptic site (Florey 1966 page 504). (This is a temporary effect and should not be confused with memory consolidation.)

In general, the presynpatic impulse sets up a transient local response in the postsynaptic cell which is not sufficient to generate an action potential (Florey 1966 page 486; Guyton 1969 page 263; Schade and Ford 1965 page 200). Large numbers of presynaptic terminals must fire within a certain interval in order that a sufficient "excitatory postsynaptic potential" be generated to initiate an action potential in the postsynpatic neuron. That is, either spatial or temporal summation of

many local potentials is required before the membrane potential at the axon hillock of the post-synaptic cell reaches threshold. John's derepressor hypothesis states that participation in reverberatory activity results in the synthesis of proteins which were previously repressed and that these proteins affect the future response characteristics of the neurons involved in the network. What presumably happens is that the derepressed proteins affect the synapses in such a way that fewer pre-synaptic firings are required for post-synaptic firing. One way this could be done is that the proteins could assemble into the post-synaptic membranes in such a way as to change their physical properties. It is known that "mechanical deformation, stretching or compressing of the membrane alters its properties sufficiently to cause a potential change" and that "stretching the membrane usually leads to depolarization" (Florney 1966 page 391). If the change in membrane potential is in the direction of the threshold then fewer pre-synaptic firings are required in order for the post-synaptic cell to reach threshold. This would allow particular reverberatory networks to be activitated under circumstances involving fewer pre-synaptic events than was initially required, which corresponds to the intuitive feeling of what memory ought to be. That is, visual memories, for instance, could be elicited without the aid of the afferent optical pathways initially involved in the establishment of these memories. The word consolidation refers to this permanent facilitation.

The discussion so far has stressed the importance of the networks involved in neural activity with very little mention of the impulse pat-

terns involved. That is, the spatial aspects have been stressed while the temporal aspects have been all but neglected. There is no doubt that the temporal impulse patterns are extremely important in perception but it is not clear that they play an important role in recall. Ungar (1970a) states that "impulse patterns undoubtedly carry information on the finer quantitative aspects of the message which are of immediate significance but are probably lost in long-term storage. It is well known that intensity discrimation, so precise when the stimuli are presented simultaneously, become insensitive with longer intervals."

Summary

In summary, we have considered several reasonable models which taken together can account for a tremendous body of experimental data concerning memory and learning. John's derepressor hypothesis can account for the coupling of the observed short-term electrical activity and the synthesis of protein which must be associated with the long-term effect of experience. Ungar has suggested a general way in which these polypeptides could be involved in establishing the neuronal networks which constitute the physiological basis of long-term memory, as well as a way in which they could be involved in the "memory transfer" phenomenon. Gally and Edelman have put forth a genetic mechanism which could account for the specificity required for Ungar's model of memory transfer without requiring the involvement of a prohibitively large number of genes. No one, of course, is suggesting that Gally and Edelman's model actually represents the mechanism for neural specificity; its importance in the context of this paper is that it eliminates the argument of those who

would say that Ungar's model cannot possibly be correct as it would require more genes than are available in the genome.

Many researchers feel that specific molecular models for memory and learning are premature because of the confusing array of data which is presently available. Perhaps they are premature; and one would certainly be foolish to suggest that any particular model represented, without a doubt, the actual mechanism involved. On the other hand, theory whether it turns out to be right or wrong, is important, and a theory which fits a very large percentage of the data, even more so. Alland (1967) in discussing behavioral genetics, points out that "good science requires feedback between theory and hard facts." It is important to have some theory to work with before collecting data so that the scientist can focus on experiments which are relevant, either strengthening the theory, disproving it, or causing it to be modified in such a way as to more closely correspond to reality. "Filling the mind with unstructured material can be just as debilitating to clear thought as armchair theorizing" (Alland 1967). Care must be taken, however, to avoid confusing the construct with reality. Neural mechanisms involved in memory and learning are infinitely more complex than the models presented here suggest; much as the reality of the hydrogen atom is infinitely more complex than the Bohr model. However, like the Bohr model, these neural models are pictorially simple and provide a visualizable basis for much of the experimental evidence. If for no other reason than this, the models outlined above serve an important and useful function.

PART 2

TRANSFER OF ACOUSTICAL PRIMING FOR AUDIOGENIC SEIZURE

CHAPTER IV

INTRODUCTION

Audiogenic Seizure

Audiogenic seizure is a unique chain of psychomotor reactions which occur particularly in rodents, in response to an acoustic stimulus of relatively high intensity. This disturbance in behavior can be divided into four distinct phases (Lehman and Busnel 1963; Henry and Bowman 1970). After a latent period of variable duration there follows an interval of extremely rapid running known as a "wild running fit." This wild running is unique to audiogenic seizures. Wild running may be followed by a second phase known as the clonic convulsive stage which is characterized by a series of rapid circular jerking motions by the rear limbs. This phase can in turn be followed by the tonic phase of convulsion. The convulsive jerking stops, and the animal is doubled over with the hind feet almost touching the mouth. Then all four limbs are slowly and completely extended and the body becomes rigid. It has been suggested that this phase represents maximum spinal activity which does not necessarily correspond to maximal brain discharge (Freston and Esplin 1961). This third phase may be followed by a fourth phase, death resulting from respiratory failure. It is likely that this fatal phase of the audiogenic seizure is the result of excitation of a distinct efferent link from that responsible for the previous phases. The convulsive behavior may be terminated after any of the first three phases with return to normal behavior in a matter of seconds.

The study of audiogenic seizure has primarily centered on certain inbred strains of mice. These strains can be classified as either susceptible or non-susceptible with respect to sound-induced convulsions with differences attributable to specific genetic backgrounds. It appears that there are several genes involved (Lehman and Busnel 1963), but as yet the specific physicochemical differences responsible for differential susceptibility are not known.

Pharmacological manipulations have provided some evidence that biogenic amines in the central nervous system are correlated to audiogenic seizure susceptibility. Schlesinger and Boggan (1968) showed that partial depletion of serotonin (5-HT) and norepinephrine (NE) with reserpine increased seizure susceptibility in the already susceptible DBA/2J mice, whereas maximal depletion either had no effect or actually protects animals against audiogenic seizures. Drugs which increased 5-HT and NE in the brain were shown to afford some protection against audiogenic seizures. In general, experiments indicate that drugs which raise brain biogenic amine levels decrease the intensity of audiogenic convulsions and that those which reduce the biogenic amine levels increase sensitivity. Again with DBA/2J mice, Schlesinger and Boggan found that seizure susceptibility decreases as levels of gamma-aminobutyric acid increase. Gamma-aminobutyric acid is an amino acid which has been implicated in the synaptic action of inhibitory neurons (Iversen 1972; Curtis and Johnson 1970). This is opposed to 5-HT and NE which generally have excitatory functions.

With certain drugs, however, paradoxical results were obtained.

For example, d. amphetamine, which reduces NE level is protective with respect to audiogenic seizure. To resolve this problem, Lehman (1967) hypothesized that the involvement of the biogenic amines in audiogenic seizure susceptibility was dependent on their release at the synaptic receptor site in a physiologically active form rather than their total brain levels. Lehman suggests that the effect of d. amphetamine on NE metabolism results in release of NE from the pre-synaptic terminal in a physiologically active form. That is, that although total brain NE level decreases, protection is obtained by an increased amount of NE at the receptor site.

These results might lead one to suspect that the differences between susceptible and resistant strains of mice might lie in endogenous brain levels of the biogenic amines. In fact, Sclesinger et al. (1965) found that seizure-susceptible DBA/2J mice did have lower brain levels of 5-HT and NE than seizure resistant genotypes. However, reduced biogenic amine levels are probably not the cause of audiogenic seizures. Most experimental results indicate that drugs which alter biogenic amine levels affect seizure susceptibility only in those strains which are already susceptible to audiogenic seizure. They cannot be used to cause seizures in resistant strains (Lehman and Busnel 1963; Schlesinger and Boggan 1968).

Acoustical Priming for Audiogenic Seizure

In 1967 it was discovered that prior intense auditory stimulation could induce audiogenic seizure susceptibility in otherwise nonsusceptible strains of mice (Henry 1967; Iturrian and Fink 1967). This phenomenon became known as acoustical priming for audiogenic seizure. Procedurally,

acoustical priming consists of exposing young mice of a normally nonsusceptible strain to intense auditory stimulation. The percentage of convulations on this first exposure is very low. However, an identical treatment at a later time can result in a very high percentage of convulsions. This phenomenon is age-specific with optimum priming and testing ages being a function of genetic background. For the C57BL/6J strain, maximum effectiveness occurs when priming is done at sixteen days of age and testing at twenty-one days of age (Henry 1967).

Normal neural functions are associated with a balance between excitatory and inhibitory processes; audiogenic seizure represents a breakdown of this balance. "Excessive neural activity leading to convulsions results either from increase in the efficiency of transmission in excitatory pathways or from decrease in effectiveness of inhibitory action" (Esplin and Esplin 1969). The seizure can be visualized as originating at a focal center of excessive excitation (oscillator) surrounded by regions of excessive inhibition. This spatial separation is accomplished by "recurrent collateral systems capable of exerting feedback control" (Spencer and Kandal 1969). The excitation is propagated by the recruitment of additional neurons to form secondary oscillations. This represents the "mediating link" (Iturrian and Fink 1969) which is responsible for the spread of the discharge to the "efferent link" and thus the physical manifestations of the seizure. The "fundamental defect in the seizure state probably reflects less an abnormality of the neuron than it reflects an abnormal utilization of the circuitry to which...(the) neurons belong. Seizure is therefore ultimately a manifestation of a system

defect although the initial trigger may be an abnormality in a number of cells" (Spencer and Kandal 1969).

In strains of mice which are nonsusceptible to audiogenic seizure, intense auditory stimulations will generally produce only normal neural functions. That is, there will be a proper balance between excitatory and inhibitory processes. However, in mice which have been acoustically primed this balance is usually not achieved and seizure can result. It is not yet clear how the acoustical priming produces this "system defect." Does the priming produce physiological damage either to the auditory apparatus or to the CNS itself, or does it produce a functional defect in the neuronal circuitry which responds to the auditory stimulus? Efforts to detect morphological changes due to acoustical priming have so far been unsuccessful, and thus it seems reasonable to believe that the effects of acoustical priming are mediated by functional changes in neuronal circuitry. If this turns out to be the case, it is entirely possible that these functional changes are produced in a manner at least similar to, and possibly identical to, the changes produced by learning during the formtion of memory as discussed in Part 1.

In addition to the possible neurological similarities between acoustical priming and learning, there are also behavioral similarities. Imprinting is an age-specific or maturationally scheduled learning situation in which brief exposure to a particular stimulus will have a dramatic effect on later behavior. This description applies equally well to acoustical priming for audiogenic seizure.

In light of these facts, it was decided to attempt to transfer

the effect of acoustical priming in a manner similar to those transfer experiments discussed earlier involving more conventional learned behavioral patterns. The suppositions upon which these experiments were based were:

1) Acoustical priming results in neural mechanisims which are in some manner similar to those initiated by a learning situation.

2) The structural changes within the brain which cause the animal to respond differently when the acoustical stimulus is presented at a later time are mediated by macromolecules (probably polypeptides).

3) The injection of these macromolecules into naive animals will alter their brain structure in a manner similar to the changes which they normally would have caused in the donor animals' brains and thereby affect the recipient animals' responses to acoustical stimuli.

The hypothesis based on these suppositions was that injection of brain extract from accoustically primed mice can increase audiogenic seizure susceptibility in nonsusceptible strains of mice.

It was hoped that injection of brain extract from primed C57BL/6J mice would have the effect of priming and that the injected C57BL/6J mice would have a high incidence of convulsion when tested at age 21 days without the previous bell normally necessary. This effect did not show up in our preliminary experiments. However, the preliminary experiments did indicate that a second bell at age 24 days would produce a higher incidence of convulsion in mice injected with brain extract from primed mice. Encouraged by these results, we developed more detailed and better controlled experiments. These experiments, which utilized both injected and non-injected litter-mate controls, will be reported here as experimental groups 1 through 8.

Following these experiments several attempts were made to demonstrate an increased susceptibility to audiogenic seizure utilizing a single test bell rung at 21 days of age. These experiments will be reported as experimental groups 9 through 12.

There are several experiments, as yet unpublished, in which information acquired at critical periods early in life has been transferred. Dr. Braud at the University of Houston has transferred imprinting in quail, and Dr. Gorbman at the University of Washington has transferred the homing of salmon (Ungar, private communication). Dr. E.J. Fjerdingstad at the University of Aarhus, Denmark, is also doing work on the transfer of acoustical priming but has not yet published his results.

CHAPTER V

PROCEDURES AND RESULTS

Subjects, Apparatus and Procedures

The subject mice were obtained from a colony which consisted of C57BL/6J females obtained from Jackson Laboratories, Bar Harbor, Maine, and their offspring. Each pregnant female was housed in an individual cage with tissue nesting material provided. Water and Purina Lab Chow were freely available. Overhead lights in the animal room were automatically turned on and off at 12-hour intervals. Auditory stimulation was delivered in a glass chamber (28.5 cm in diameter and 26.5 cm deep) with a wooden top. Fastened to the underside of the top was a 114 db (for entire spectrum; 105 db for the octave centered at 8 KHz) electric doorbell (6.5 cm in diameter).

All procedures involving the mice were carried out during the fourth or fifth hours of the light cycle because of the circadian nature of audiogenic seizures (Schreiber and Schlesinger 1971).

At the age of 16 days each donor litter was removed from its mother and the mice were individually taken into a separate room containing the sound chamber. They were then placed in the sound chamber and the electric bell was rung for 30 seconds. After each animal in the litter had been primed, the litter was returned to its mother. Donors were sacrificed by decapitation. Within 20 seconds their brains were removed and frozen in liquid nitrogen. These were stored in a freezer at

-20°C until 43 brains had been accumulated. The brains were then homogenized in a blender with 30 ml of deionized distilled water and put in dialyzing bags. These were dialyzed against 600 ml of deionized distilled water for two days with continuous stirring for the first day. After lyophilization, the residue was redissolved in 5.75 ml of deionized distilled water.

Experimental litters were removed from their mothers and approximately one-half of each litter was injected intraperitoneally with 0.25cc of primed brain extract (extract from approximately two donor mice). The remainder of the litter was either not injected or was injected with 0.25cc of a control solution. After injection the litters were returned to their mothers. At 21 days of age the experimental mice were individually taken to the sound chamber and exposed to the bell in exactly the same way as the donor mice had been primed. This procedure was repeated at age 24 days.

The experiments involved several permutations of the time after priming at which the donors were sacrificed and the age at which the recipients were injected. The experimental groups are represented in Table 1. Those groups injected at age 21 days were injected two hours before the first test bell. The brain extract from non-primed mice was prepared in exactly the same manner as the brain extract from primed mice.

Several attempts were made to demonstrate an increased susceptibility to audiogenic seizure utilizing a single test bell run at age 21 days. Donors, recipients, and controls were as in experimental group 7. That is, donors were sacrificed five days after priming, and recipients

Table 1. Experimental Groups 1 Through 8

Experimental group	Donor priming-sacrifice interval (days)	Injection age (days)	Number of recipients N	Controls	
				Control injectant	N
1	5	20	15	none	17
2	5	21	6	none	9
3	3	19	10	none	13
4	1	17	7	none	9
5	5	20	21	non-primed brain extract	21
6	5	20	18	physiological saline	22
7	5	18	19	physiological saline	19
8	5	21	18	physiological saline	20

Table 2. Experimental Groups 9 Through 12

Experimental group	Number of recipients	Number of controls	Treatment
9	4	4	The recipients were injected with extract from the brains of four donor animals (twice the normal dose).
10	12	12	Both recipients and controls were injected with 2.5 mg/kg reserpine one hour before test bell.
11	17	19	Both recipients and controls were injected with 1.0 mg/kg reserpine one hour before test bell.
12	23	22	Procedurally identical to experimental group seven.

and controls were injected at age 18 days, the controls being injected with physiological saline. These experimental groups are represented in Table 2.

Results

In experimental groups 1 through 8, the incidence of response to the first test bell (bell at age 21 days) was low in both recipients and controls with 12 out of 114 recipients exhibiting the wild running phase (Lehmann and Busnel, 1963) and 5 out of 130 control animals exhibiting the wild running phase. The group showing the most effect was group 7 with 5 out of 19 recipients as opposed to 1 out of 19 controls exhibiting wild running. Group 7 was injected three days before the first test bell whereas most of the other groups were injected either one day or two hours before the first test.

At the second test bell (bell at age 24 days), however, the effect of the extract was much more apparent as seen in Figure 4. The most stiking results were obtained using donors sacrificed five days after priming and recipients injected at age 20 days (groups 1, 5 and 6). In these groups the control animals were not inejcted, were injected with extract from non-primed mice, and were injected with physiological saline respectively.

We do not wish to suggest that using donors sacrificed five days after priming and recipients injected at age 20 days represents the optimum procedure for demonstrating the effects of injection of brain extract from acoustically primed mice. The number of possible sacrifice-injection-testing permutations is large and much more work is needed.

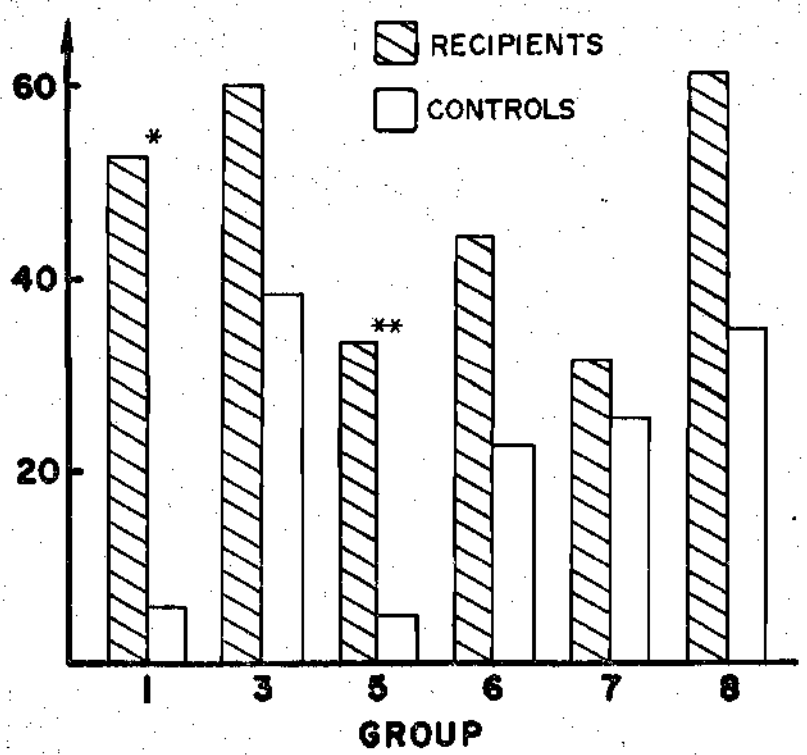

Figure 4: Percentage of Mice Exhibiting Tonic or Fatal Convulsions Upon Exposure to Bell at Age Twenty-four Days.
* P = .0041 using the exact method.
** P = .0225 using the exact method.

Group 4 consisted of 16 mice from two litters of very small mice. Of these, 13 had fatal convulsions on the second test at 24 days of age. A high incidence of convulsion was noted in some of the other litters consisting of small mice (see Chapter VII). To avoid this effect mice were weighed at 20 days of age and only those litters averaging eight grams or more per mouse were used in group 5. Mice were weighed at 18 days of age and only those averaging over seven grams per mouse were used in groups 6, 7, and 8.

Group 2 had one fatal convulsion among the six recipient mice and two fatal convulsions among the nine litter-mate controls. This group is not shown in Figure 4.

An attempt was made to determine if the sex of the experimental animal was a factor in either the transfer results or the acoustical priming results in general. The sex of 76 mice injected with primed brain extract and 581 mice either injected with a control injectant or not injected was recorded during the test at 24 days of age. The percentage of males showing maximum convulsion was virtually identical to the percentage of females showing maximum convulsion in both of these groups (χ^2 tests yeilds $P \simeq 0.80$ and 0.75 respectively), and therefore it was concluded that no sex difference exists.

The large variability in response to the second bell at 24 days of age, caused in part by variations in subject weight, makes non-litter-mate comparisons difficult. For this reason Figure 4 provides no information which would allow the different control procedures to be compared directly with one another.

Experimental groups 9 through 12 represent attempts to demonstrate transfer of the effect of acoustical priming utilizing a single test bell rung at 21 days of age. In addition to the litter-mate controls used in these experiments, a separate experiment involving 525 untreated C57BL/6J mice was conducted. These mice were exposed to a single bell at 21 days of age and their responses recorded with the following results: 3.8% wild running only; 0.76% clonic but not tonic convulsions; 0.38% tonic but not fatal convulsions; and zero fatal convulsions.

In experimental group 9 the recipient mice died within 24 hours of injection probably due to the highly concentrated nature of the 0.25cc injection. This experiment was promptly discontinued.

In experimental groups 10 and 11 injections of reserpine were given one hour before the test bell. (This procedure was suggested to me by Dr. Iturrian, University of Georgia.) There is experimental evidence that reserpine causes an increase in audiogenic seizure susceptibility in those strains which are already susceptible while having little effect on non-susceptible strains such as the C57BL/6J (Lehman and Busnell 1963; Schlesinger and Boggan 1968). It was hoped that the reserpine would amplify any increased susceptibility to audiogenic seizure which might be caused by brain extract injection while having little or no effect on the saline injected controls.

In experimental group 10, which was given 2.5 mg/kg of reserpine, there was no indication of even a wild running fit in either the recipient or control animals. These animals appeared heavily sedated with only about half of them showing any movement at all during testing.

In experimental group 11, which was given 1.0 mg/kg of reserpine, there was an apparent increase in susceptibility in both the recipient and the control animals. Among the 17 recipients there were three wild runnings, one clonic convulsion, and one fatal convulsion. However, among the 19 controls, where little or no convulsive activity was expected, there were three wild runnings and one fatal convulsion. These results can be compared to population response measurements given earlier which show 4.94% wild running fits or greater on exposure to the first bell at 21 days of age.

Experimental group 12 was an attempt to replicate or perhaps improve on, the first bell results obtained with experimental group 7. Experimental groups 7 and 12 received their brain extract and saline injections at 18 days of age, three days before the first test bell. In experimental group 7, five of the 19 recipients had wild running fits upon exposure to the first test bell whereas only one of the 19 controls had a wild running fit. In experimental group 12, three of the 23 recipeints and one of the 22 controls had wild running fits. Combining the two groups gives eight out of 42 (19.5%) recipients and two out of 41 (4.9%) controls with wild running fits. Again, these results may be compared with the population measurements given earlier.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Experimental groups 5 and 6 are procedurally the same except that group 5 controls were injected with brain extract from non-primed mice and group 6 controls were injected with physiological saline. If we assume that these two injectants are not different from one another with respect to their ability to effect audiogenic seizure susceptibility we may combine these two groups as is shown in Figure 5. Additionally, if we assume that intraperitoneal injection in general does not effect audiogenic seizure susceptibility, then we may combine groups 1, 5 and 6. This is also shown in Figure 5.

Group 5 is perhaps the critical group in these experiments because this group indicates that brain extract per se is not responsible for the increased susceptibility to audiogenic seizure seen in the other experimental groups.

We feel that the reported results show that injection of brain extract from acoustically primed mice can increase the susceptibility of C57BL/6J mice to audiogenic seizure. The preliminary experiments and experimental groups 1 through 8 represent data from 274 mice. Of these 274 mice, 132 were injected with extract from primed mice and 142 were control mice. The incidence of tonic convulsion or greater among the 132 recipient animals was 47% whereas it was 25.3% among the 142 control animals. These results are shown in Figure 5. A χ^2 test indicates a level of significance $P < .001$.

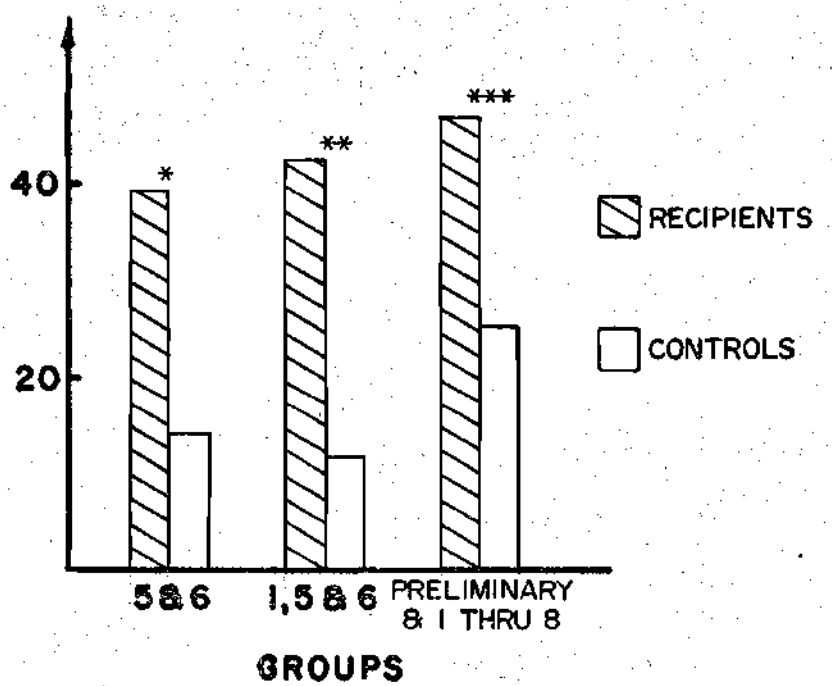

Figure 5: Percentage of Mice Exhibiting Tonic or Fatal Convulsions Upon Exposure to Bell at Age Twenty-four Days.
* $.02 < P < .025$ using χ^2 with Yates correction.
** $P < .001$ using χ^2 with Yates correction.
*** $P < .001$ using χ^2.

We also feel that these results give reasonable support to the supposition that acoustical priming results in neural mechanisms which are in some manner similar to those initiated by a learning situation and at the very least indicate that the effects of priming are mediated by CNS mechanisms.

Attempts to demonstrate a clear effect associated with the first bell were generally disappointing. Experimental groups 7 and 12 showed a small but consistent effect. Comparison of the 42 recipient animals to the population data using a χ^2 test with Yates correction yields $P < .001$. Although this is highly significant, the procedure is probably not worth pursuing because of the small percentages (19% vs. 4.9% for population) involved. It is possible, and we think probable, that a more appropriate procedure will demonstrate a more dramatic effect on the first test bell. In fact, Dr. Iturrian* at the University of Georgia has since been able to demonstrate greatly increased susceptibility to audiogenic seizure (90%) on the first test bell, resulting from injection of primed brain extract in CF#1 (another non-susceptible strain) mice with the use of reserpine in a manner similar to that used in experimental groups 10 and 11.

The positive results reported here have important application in at least two distinct areas of research: "memory transfer" and acoustical priming for audiogenic seizure.

First with respect to "memory transfer" research, these experi-

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*Private communication

ments represent the first case of transfer of one-trial learning. The outstanding feature of the "memory transfer" experiments is that they enable us to detect the chemical correlates of learned information in the brain of the donor animal (Ungar 1970a). Therefore, the transfer of one-trial learning is particularly important, since it provides a unique opportunity to study the kinetics of formation and disappearance of the "active factor" in the donor animal's brain. Additionally, acoustical priming for audiogenic seizure has practical advantages as a behavioral procedure for transfer studies in that donor "training" is such a simple procedure.

With respect to acoustical priming for audiogenic seizure, there are currently two schools of thought on the mechanism involved: first that it is essentially a mechanical phenomenon involving the middle or inner ear, and secondly that the effects of priming are mediated by central nervous system mechanisms. Our positive transfer results can be considered as evidence that CNS mechanisms are involved. Additionally these experiments may shed light on the physiological mechanism of the audiogenic seizure itself and therefore have application to the study of sensory induced seizures in humans.

Eventually, the chemical nature of the "active factor" in the extract will have to be determined to see how it compares with the behaviorally active peptides which have been isolated, analyzed and synthesized by Ungar from "memory transfer" experiments involving more conventional learned behavioral patterns. This should give us a better idea as to the physiological similarity between priming and normal learned behavior.

Henry (1970) reports that amnesic agents such as hypothermia, ECS, and inhibitors of brain protein synthesis do not effect the priming results. The positive results of the transfer experiments reported here suggest that the effect of amnesic agents on acoustical priming should be studied in more detail.

CHAPTER VII

EFFECT OF WEIGHT VARIATION ON ACOUSTICAL PRIMING RESULTS

While working with experimental groups 1 through 4 it was noticed that smaller mice had a much higher percentage of tonic or greater convulsions on the second exposure to the bell (i.e., the bell which was rung at 24 days of age after an initial bell at 21 days). This effect involved both recipient and control animals. As a result, mice involved in subsequent transfer experiments were required to meet some minimum weight standard as stated previously.

An experiment was conducted which was aimed at substantiating the effect of size on acoustical priming for audiogenic seizure results. Mice were weighed at 20 days of age and exposed to the bell at 21 and 24 days of age in exactly the same manner as described previously. The results of the test at 24 days of age are given in Table 3.

The distribution of mice in the different weight categories does not represent the population distribution for our colony. Mice which could not meet the weight criterion for the transfer experiments were utilized in addition to those litters which were originally intended for this experiment.

It is well known that the effectiveness of acoustical priming for audiogenic seizure in C57BL/6J mice is strongly dependent on the age at which the priming occurs (Henry 1967). The experiment just reported demonstrates that the weight of the mouse at a particular age is also a

Table 3. Weight Dependent Seizure Susceptibility

weight per mouse in grams	fraction with tonic convulsions or greater	percentage
< 6.0	68/83	81.9%
≥ 6.0 but < 7.0	112/185	60.5%
≥ 7.0 but < 7.5	64/119	53.8%
≥ 7.5 but < 8.0	5/29	17.2%
≥ 8.0 but < 9.0	9/51	17.6%
≥ 9.0	9/41	21.9%

very important parameter affecting the acoustical priming results. It is doubtful that these two observations stem from independent phenomena but rather that they are both manifestations of a single physiological condition: degree of neural maturation. Henry (1967) reports that C57BL/6J mice primed at 18 days of age and tested three days later show 67% tonic or greater convulsion. It is conceivable that 21 day old mice in the six to seven gram range are neurologically similar to the 18 day old mice in Henry's paper.

The variance in effectiveness of acoustical priming for audiogenic seizure is not entirely due to age and weight differences. Litters of the same age and weight can vary considerably in their response to acoustical priming. For example, in the data reported on the effects of weight, five of 20 mice in the 7.5 to 8.0 gram per mouse category exhibited tonic convulsions or greater. However, four of these five were in a single litter of six mice. Whether this is the result of physiological differences or of subtle experimental differences is not known, but it points out the necessity of using litter-mate controls and of alternating recipient and control animals during testing as was done in the transfer experiments reported here.

APPENDIX A

DETAILS OF THE EXPERIMENTAL EVIDENCE FOR MULTIPHASE MEMORY

Electroconvulsive Shock

Most of the evidence for a short-term memory phase as distinct from the permanent long-term phase comes from electroconvulsive shock (ECS) data in humans and laboratory animals. These results indicate that ECS can cause permanent retrograde amnesia (RA) and that there is a temporal gradient associated with the degree of amnesia which seems to be a function of the material to be remembered, the severity of the ECS, and the species involved. Most researchers feel that ECS interferes with the mechanisms by which long-term memory is established rather than disrupting a memory which has already been consolidated.

In 1937, Bini and Cerletti introduced electroconvulsive therapy for treatment of psychoses. Cases of apparent retrograde amnesia were frequent, and Zubin and Barrera (1941) attempted to verify these reports experimentally. They found that ECS abolished savings in the relearning of lists of paired associates originally learned before treatment. They also found that the severity of impairment was inversely related to the learning-ECS interval.

The early results which purported to have demonstrated retrograde amnesia in animals as a result of ECS were criticized for a number of reasons. One objection concerned the fact that much of the behavioral changes which were used to "demonstrate" the amnesic effect of ECS could have been

the result of a punishing effect. In order to answer this criticism, a one-trial passive avoidance situation was developed in which the animal was placed on a platform, and upon stepping down from the platform received a foot-shock. Some of the subjects received ECS within seconds after the foot-shock. If ECS exerted its effect by punishing the subject, this procedure should have increased step-down latencies on a retest 24 hours later. Instead, step down latencies were significantly less than for the control group which had received only the foot-shock the previous day (Jarvik 1970).

Others suggested that the effect of ECS was proactive rather than retroactive. In examining this possibility, Kopp et al. (1967) measured step-through latencies for mice given a mild foot-shock punishment. They found that a single ECS administered before retest did decrease retest step-through latencies, but only when the ECS was administered eight hours or less before retesting. This separated the proactive disinhibitive effect of the ECS from the retroactive effect, since retest occured at least 23 hours after the ECS. It would be extremely difficult to explain amnesia measured six weeks after treatment in terms of a proactive effect. Although there is now general agreement on the fact that ECS produces retrograde amnesia, it is far from unanimous (e.g., Deutch 1969).

The concept that ECS exerts its effect by disrupting the consolidation process was challenged in a paper by Misamin et al. (1968). They reported that ECS given 24 hours after training could effectively disrupt memory, provided the memory trace was reactivated immediately before the ECS was administered. Presumably consolidation had already taken place

and the pertinent physical phenomenon was the reverberating electrical activity rather than the consolidation which was initially associated with it. Dawson and McGaugh (1969) attempted to replicate Misamin's results, and were not able to reproduce the essential feature. Their results support the view that ECS disrupts memory consolidation and has no memory disrupting effect on a recently reactivated memory trace.

In studying the amnesic effects of electroshock, most investigations have used sufficient current to produce convulsion. However, McGaugh and Alpern (1966) were able to show that the convulsion produced by electroshock stimulation seemed to be unnecessary for the occurrence of amnesia. Anesthesia with ether prevents the electroshock convulsion, but electroshock stimulation given anesthesized mice was effective in causing amnesia. In this experiment, the ether anesthesia by itself produced no amnesia. Also, Jarvik and Kopp (1967) found that subconvulsive transcorneal electrical stimulation (3 ma for 0.2 seconds) was effective in producing RA.

Permanance of ECS Induced Retrograde Amnesia

In order for ECS results to be considered as evidence for a short-term memory phase, the disruptive effects of ECS must be permanent in at least some cases. Chevalier (1965) found that for a one-trial training procedure ECS produced interference with retention which persisted undiminished for 30 days. However, Zinkin and Miller (1967) found that although ECS given immediately after one-trial avoidance learning produced significant amnesia 24 hours later, the effect had largely disappeared 48 hours and 72 hours after treatment. They suggest the possibility that the recovery may be due to learning which would depend on there being some

minimum retention of the traumatic properties of the situation. This interpretation is strengthened by Luttges and McGaugh (1967), who found no such effect if separate groups were tested at different time intervals, and therefore each subject was tested only once. The amnesia was shown to last at least one month, even though control animals still remembered the training at that time. Similar results were found by Peck and Herz (1967) who found apparent recovery of memory in mice 72 hours after ECS if they were tested 24 and 48 hours after treatment, but no recovery when tested only 72 hours after training.

Another factor effecting the permanance of ECS induced amnesia is the severity of the ECS treatment. Miller (1968) found increased retention 48 hours after treatment with rats given a 35 ma ECS but no increase in rats given a 100 ma ECS. He also noted that partial recovery was limited to those animals whose ECS had come at a time greater than 3.1 seconds after training. Rats given a 35 ma ECS 3.1 seconds after training showed no recovery of memory.

Geller and Jarvik (1970) were able to show the permance of retrograde amnesia resulting from immediate ECS in a one-trial passive avoidance situation over a 6-week testing interval. They suggested that many of the recovery results could be explained as recovery from the proactive effects and that in these cases there was no retroactive effect.

Temporal Gradient of Retrograde Amnesia Induced by ECS

The existence of a temporal gradient for ECS data involving experimental animals is a generally accepted fact. That is, almost all investigators have found that the measure of retrograde amnesia is greater the

shorter the training-ECS interval and that beyond a certain interval, ECS is no longer effective in producing RA. This has obvious theoretical importance in that it must reflect some changing process within the organism and may be interpreted to mean that the ECS disrupts a consolidation process and that the interval between training and ECS treatment is a relative measure of the amount of consolidation which has already taken place. Of additional theoretical importance is the fact that the amnesic gradient is permanent. Geller et al. (1970) used training-ECS intervals of ten seconds, two minutes, and three hours to establish the gradient and found that is persisted with retest intervals up to four weeks.

A factor which greatly complicates the theoretical interpretation of the ECS data is the wide discrepancies in the published intervals after training for which ECS can produce demonstrable amnesia. These results range from a few seconds to many hours. Chorover and Schiller (1965), using a one-trial passive avoidance paradigm, found that 35-50 ma ECS of 0.2 seconds duration was effective in producing RA only if administered 10 seconds or less after training. Chevalier (1966) found that 82 ma ECS of 0.3 seconds duration was effective with a 30-second training-ECS interval but not with a five-minute interval. Pinel (1969) used a one-trial appetitive learning situation and tested the rats at intervals of 10 seconds, one minute, 10 minutes, one hour, and 24 hours after training. The testing indicated no significant differences in performance. In a separate experiment, rats were given ECS at these same intervals and were tested 24 hours later. Statistically significant retention deficits were produced by ECS given 10 seconds and one minute after training whereas,

those given ECS 10 minutes or more after training were indistinguishable from the no-ECS group. The ECS was 60 ma for 0.5 seconds duration. On the other hand, Kopp et al. (1966) using only 15 ma for 0.2 seconds showed a temporal gradient with a training-ECS interval from 0.5 seconds to six hours, with the six-hour data being significantly different from controls. In an extreme case, Robustelli et al. (1969) found that by extending the cut-off latency, they could demonstrate a RA effect with a training-ECS interval of 23 hours. In this experiment they used 15 ma for 0.2 seconds. This paper may explain in part why other experimenters have obtained shorter times for the maximum effective training-ECS interval. Mice were given a mild foot-shock after stepping down from a platform, and step-down latencies on retest one week after training were used as a measure of amnesia. The cut-off latency was set high (1200 seconds), and measurable amnesia was produced by the 23 hour training-ECS interval. The average step-down latency was about 450 seconds as opposed to about 700 seconds for no-ECS control group. However, if the cut-off latency had been set at 300 seconds, which at first seems to be a perfectly reasonable time, only the 10-minute training-ECS interval would have indicated an effect, with the 30-minute and all longer intervals being indistinguishable from controls.

It would appear that the task involved may play a significant role in determining the temporal gradient of RA. Herz (1967) found that appetitive learning is more completely attenuated than is an aversive task with short training-ECS intervals, but for longer intervals the reverse is true. It is tempting to interpret these results as indicating a dif-

ference in consolidation time for appetitive and aversive learning, but results by Chorover and Schiller (1966) and Geller and Jarvik (1970) indicate that caution is in order.

Chorover and Schiller attempted to determine if performance deficits produced by ECS with long training-ECS intervals were due to factors other than retroactive interference with memory. They were convinced that ECS could produce RA but felt that effective long training-ECS intervals were not consistent with the disruption of consolidation mechanism. It was hypothesized that what appeared as passive avoidance in many experiments was actually a consequence of the locomoter inhibition component of a nonspecific conditioned emotional response (CER). Hunt and Brady (1951) had demonstrated that ECS interferred with the CER, and Chorover and Schiller felt that this could explain the existence of long termporal gradients of retrograde amnesia. They were able to show that ECS decreased the locomotor inhibition, which is characteristic of the nonspecific CER, and concluded that it was this increased exploratory activity rather than a prolonged RA which causes the apparent memory impairment in many of the passive avoidance experiments. Furthermore, Chorover and Schiller were able to reproduce the experiments which had resulted in long temporal gradients of RA, and then by eliminating the CER-inducing procedures the long-term disruptive effect of the convulsion was also eliminated.

In related work involving passive avoidance, Geller and Jarvik (1970) studied the relationship between the amnesic gradient produced by ECS and the incubation curve. Incubation was first described in one-trial learning situations by McMichael (1966) and Pinel and Cooper (1966a, 1966b)

and refers to the fact that performance is not maximum immediately after training, but gradually increases to some maximum value and then begins to drop off. With a one-trial step-through apparatus with a 30-second delayed foot-shock, Geller et al. (1970) were able to obtain an incubation curve with maximum at 48 hours. In separate experiments Geller and Jarvik were able to compare incubation curves and ECS gradients for several different training conditions and at many different times after training. They were not able to separate these two curves. In one experimental situation they found that the incubation curve was not a simple monotonic function, but instead shows a peak at around five minutes after training; that is, the incubation curve is a biphasic curve. Remarkably, the curve for the subjects given ECS at the same time intervals and tested 24 hours later showed exactly the same biphasicity.

If the incubation curve results from time dependent CER, then the results of Geller and Jarvik are explained by the results of Chorover and Schiller. It is interesting to note that there is no incubation effect in the previously mentioned appetitive experiments of Pinel (1969) in which he reported that only short training-ECS intervals were effective in producing RA. This is also consistent with the results of Herz (1967) who found that long training-ECS intervals were effective in an aversive task but not in a appetitive task.

An additional complication of interpreting temporal gradients of RA produced by ECS is pointed out in a paper by Cherkin (1969). His experiments indicate that weak amnesic treatment may fail to block memory consolidation but will slow its rate. Therefore, post-treatment consoli-

dation would inflate the retention scores measured 24 hours later and lead to variably shortened consolidation times.

Factors Other Than Training-ECS Interval Which Effect ECS Results

There is a growing body of evidence which suggests that the time between learning and ECS is not by itself the only determinant of the amnesic effect of ECS (Miller et al. 1969). The effect of different ECS currents has already been mentioned with respect to the permanence of the induced amnesia. The duration of the shock produced no measurable effect in that experiment (Miller 1968). However, McGaugh (1966) found that extending the duration of the ECS treatment could affect the training-ECS interval which would produce demonstrable RA. He found that ECS produced by 15 ma for 0.2 seconds did not significantly affect retest performance when administered one hour after training. However, RA resulted with training-ECS intervals as great as three hours if the duration of the treatment was extended to either 0.4 or 0.8 seconds. In contrast to Miller's results, McGaugh found no measurable effect of the magnitude of the current provided it was sufficient to produce convulsion. McGaugh also noted that different durations of electroshock stimulation have markedly different effects on electrophysiological activity of the brains of mice.

The learning-ECS interval for which amnesia is observed is apparently also a function of the events occurring during that interval. Miller et al. (1969) found that ECS given three seconds after learning of a one-trial passive-avoidance task normally produced only partial amnesia. However, when the animals were subjected to a flashing light during the same

learning-ECS interval, a more complete amnesia resulted. The light alone had no disruptive effect on subsequent performance.

Foot-shock intensity is another factor affecting the effective learning-ECS interval in the one-trial passive-avoidance situation. Ray and Bivens (1968) were able to produce RA with a learning-ECS interval of 160 seconds if the foot-shock was 0.3 or 1.5 ma but not if it was 2.8 ma. However, with a learning-ECS interval of 10 seconds they obtained RA with the same ECS treatment for all three foot-shock intensities. These results suggest that reinforcement magnitude is directly related to the speed of consolidation. Similarly, an increase in the duration of the foot-shock was found, within limits, to correspond to a decrease in the interval at which RA could be produced (Chorover and Schiller 1966).

Another factor affecting ECS results is brain temperature. It is reasonable to suspect that the biochemical processes responsible for consolidation proceed at a slower rate at lower temperatures. Agranoff (1970a) reports that goldfish cooled 10°C for two hours following training remained susceptible to ECS, whereas normally ECS has no amnesic effect when administered at this time. Gerard (1955) reported that hypothermia extends the period during which ECS can produce performance deficits in hamsters. He found that ECS at one hour could produce the same effect as ECS at 15 minutes if coupled with hypothermia. Temperature effects must be taken into account when comparing results obtained from mice with results from goldfish.

Drugs

Although it now seems likely that reverberatory neural activity is

the basis for short-term memory, it is clear that long-term memory cannot be attributed to enduring reverberation. Long-term memories have been shown to survive treatment which severely disrupts electrical neural activity (e.g. ECS, spreading depression, and cathodal polarization). The fact that some memories last a lifetime and are extremely stable requires changes to occur in the matter of which the brain is constructed and therefore almost certainly involves protein synthesis.

Katz and Halstead (1950) proposed that macromolecules (nucleoproteins) were responsible for the formation of memory. The first important experimental evidence for macromolecular involvement in learning was provided by Hyden and Egyhazi (1962, 1963, 1964). They studied RNA changes in isolated neurons and glia and demonstrated increased RNA synthesis and base ratio changes associated with prolonged training experiences. Hyden (1967, 1969) emphasizes that while neural activity alone can increase the amount of RNA in the neuron, altered base rations are associated with learning.

The increase in the synthesis of RNA with a learning situation has been confirmed by Zemp et al. (1966, 1967), and by Shashua (1968). Machlus and Gaito (1969), using successive competition hybridization, obtain results which suggest the synthesis of "unique" RNA species during learning.

Hyden and Lange (1965) found that the base ratios of the newly synthesized RNA in the brains of rats being trained was dependent on learning curve position. They concluded that genetic activation occurs early during a learning process. "Thus, an acute learning situation may

select parts of the genome which become activated. The primary gene products, in the cases analyzed, adenine-uracil-rich RNA, are followed by a ribosomal type of RNA which takes over the long-term synthesis of protein necessary to sustain the neural functions of the new behavior." In an experiment in which rats were forced to reach for food with the nonpreferred front paw, RNA from cortex contralateral to the trained paw occurred in greater quantities per neuron than in the ipsilateral cortex and with a different base composition. The control ipsilateral cortex (which is not necessary to transfer of handeness) has neuronal RNA in amounts and composition not distinguishable from that in either cortex of untrained animals.

Inhibition of Protein Synthesis

In a variety of animal species and a number of behavioral tasks, inhibition of protein synthesis before or shortly after learning appears to interfere with retention of the learned response. This has been interpreted by most investigators to be due to an interruption of events which would normally lead to long-term memory storage.

Barondes (1970) and his co-workers injected mice intracerebrally with 20 mg of acetoxycycloheximide (AXM) and when protein synthesis was inhibited by 95% or more, trained them to escape shock by choosing either the left or right arm of a T-maze. The AXM-injected mice learned to a criterion of three out of four consecutive correct responses in the same number of trials as a saline injected control group. When tested three hours later, both groups showed normal memory. These results indicate that the ability to synthesize protein is not necessary for either learning

or short-term memory. However, when tested from six hours to seven days after training, retention was markedly impaired in the AXM-injected group. Protein synthesis had returned to normal four days after injection so that the effect at seven days was not due to the inability to synthesize protein. Barondes also found that subcutaneous injections of 240 mg of AXM from five hours to five minutes before training produced performance deficits in mice when tested seven days later. Injections immediately before, immediately after, and five minutes after training were also effective, but less so, and post-training injections at 30 minutes and 24 hours were not distinguishable from controls. In the experiments involving subcutaneous injection, approximately 90% of the cerebral protein synthesis was inhibited within 10 to 15 minutes of the injection.

If the mice were trained to a criterion of nine out of 10 consecutive correct left or right responses rather than three out of four, the AXM injections had no effect on subsequent memory. Apparently the amnesic effect of the inhibitors could be obscured by overtraining, perhaps because of the small amount of residual capacity to synthesize protein. Cohen and Barondes observed a similar effect with a more difficult task involving visual discrimination. Here, pretrial AXM injections produced a later decrease in retention if the training creterion was nine out of ten correct responses but not if a criterion of 15 out of 16 was used.

In an attempt to determine the generality of the AXM effect, a task motivated by thirst rather than escape from shock was used. The results in the appetitive task were identical with those in the aversive task (Cohen and Barondes 1968).

Flexner (1968) indicated that the amnesic effects of pretrial AXM injections may not be permanent. Mice were injected intracerebrally and five hours later trained in a maze. He found a period after training in which memory was retained, an intermediate period during which the mice showed less retention than controls, and a final period during which expression of memory returned. In essentially all mice, memory returned to a high level around 60 hours after training, which was at least 20 hours after protein synthesis had returned to normal. These results closely resemble those relating to the permanence of the amnesia induced by ECS and quite possibly can be explained in the same way. If Flexner's AXM injections, in possible combination with a degree of overtraining, merely slowed down the consolidation rate rather than blocking consolidation, then his results might be expected. That is, the decreased rate of consolidation could result in the level of consolidation sufficient for behavioral modification being reached well after the short-term memory had dropped below the behavioral threshold.

Using post-trial injections of 150 mg/kg of cycloheximide in a one-trial passive-avoidance task and testing one week later, Geller and Jarvik (1970) were able to obtain a temporally graded retention deficit similar to those obtained using ECS. The retention gradient extended up to 30 minutes after training.

Working with goldfish, Agranoff (1970) and his co-workers were also able to obtain a temporal gradient. The goldfish were give 0.1 to 0.2 mg of AXM intracranially. If the injection was given immediately after training, there was a profound memory loss on retesting three days later. How-

ever, if the injection was given a few hours after training, there was little or no effect on 72-hour memory. Agranoff concluded that memory consolidation in the goldfish is complete within three hours after training. However, he observed that performance declined gradually after injection, and that it took almost three days for the memory to effectively disappear. Performance was still good 24 hours after injection. Apparently short-term memory can survive for quite a long time in goldfish and obscure the effect of the inhibition of the long-term phase.

Puromycin is another inhibitor of protein synthesis which has been used extensively in memory research with results generally the same as for AXM (Agranoff 1970a). Cohen et al. (1966) and Cohen and Barondes (1967) were able to show that puromycin not only inhibited cerebral protein synthesis but also produced abnormalities in cerebral electrial activity and increased the propensity to seizure. Burkhalter (1963) reports that puromycin alters cholinesterase activity and this may be the mechanism of interference with the electrical activity. For these reasons the puromycin results are much more difficult to interpret. AXM, on the other hand, apparently does not interfere with cerebral electrical activity (Barondes 1970), but the possibility that it interfers with memory because of some unknown side effect, rather than by inhibition of protein synthesis, cannot be absolutely ruled out.

It is possible that amnesic drugs may exert their influence either by an effect in addition to disrupting consolidation or by an effect other than the disruption of consolidation. This is suggested in a paper by Davis and Kliger (1969), which gives similar results to those obtained

by Misanin et al. (1968) using ECS. Davis and Kliger found that amnesia could be obtained 24 hours after training with KCl, puromycin, and AXM if goldfish are replaced in the intertrial environment for a brief period just prior to injection. That is, apparently the injection could act on the reactivated memory trace after consolidation should have taken place.

Effects of Drugs Other Than Inhibitors of Protein Synthesis on Memory

Cohen and Barondes (1966) used intracerebral injection of actinomycin-D in mice to inhibit RNA synthesis by 94-96%. Mice were then trained in one-and two-choice mazes and tested up to four hours after training. The actinomycin-D group was indistinguishable from the control group. Because of systemic toxicity which became apparent eight to ten hours after the administration of actinomycin-D in mice, it was difficult to make concrete statements concerning the effect of actinomycin-D on the long-term memory of mice. Agranoff et al. (1967) found that when 2 mg of actinomycin-D were injected in goldfish intracranially immediately after a training session, the formation of long-term memory of shock avoidance training was blocked. Tests four days after training showed injections at zero and one hour were effective in blocking the formation of long-term memory but injection three hours after training was not. Agranoff suggested that in experiments with mice, loss of long-term memory was obscured by residual short-term memory. Recall that Barondes (1970) showed behavior at least three hours after training was uneffected by doses of AXM, which blocked formation of long-term memory.

Squire et al. (reported in Cohen 1970) found that lower doses of actinomycin-D caused less toxicity and allowed long-term memory in mice

to be assessed. When tested 24 hours after training, the drug-treated mice showed little retention whereas saline-injected mice performed well.

Codish (1971) found that injection of actinomycin-D blocked the formation of long-term memory in chicks. Injections given 30 minutes after imprinting were effective when tests were given three and six days later, whereas injections given 24 hours after imprinting were not.

Another inhibitor of RNA synthesis, 8-azaguanine, has been used to interrupt consolidation of long-term memory. Dingman and Sporn (1961) found that intracisternal injection of 8-azaguanine interferred with the ability of rats to learn a new maze, although it did not interfere with the ability to recall and perform a previously well learned maze.

Possible Links Between Long- and Short-Term Memory

As discussed earlier, Agranoff (1970) found that while either AXM or puromycin administered immediately after training could effectly block formation of long-term memory in goldfish, performance was not immediately affected, but rather decayed gradually over a three day period. As a possible explanation for this prolonged decay of short-term memory, he suggested the possibility that protein synthesis might be required for the decay of short-term memory as well as for the formation of long-term memory. A test for this hypothesis was to see if repeated injection of AXM could prevent the decay of short-term memory. One group of goldfish was injected with AXM immediately following training; another group was injected at 0, 24, and 48 hours after training. When tested three days after training the 0, 24, 48 hour group performed significantly better than the single injection group. Arganoff feels that these results are

support for the concept that short-term memory is "converted" to long-term memory.

If Agranoff's suggestion, that inhibition of protein synthesis delays the decay of the temporary phase of memory, is correct, it seems reasonable that the temporary holding phase is affected, rather than the reverberatory phase. Cytoplasmic concentrations could be affected by protein inhibition effects on either cell membrane permeability or cytoplasmic metabolism. It is more difficult to see how inhibition of protein synthesis could prolong the reverberatory phase.

Another observation which may have a bearing on the relationship between short- and long-term memory is that memory consolidation is moved forward in time if goldfish are maintained in the training environment instead of being returned to the home tanks (Davis and Agranoff 1966; Davis 1968). If left in the training tank for as long as three hours after completion of training, the period of susceptibility to protein synthesis inhibition is correspondingly lengthened. It appears that memory fixation is blocked when the organism is in an environment associated with a high level of stimulation.

Memory Transfer

It is unfortunate that the term "memory transfer" has gained such wide usage, since it implies something other than what is probably happening. It also has a sensational ring to it, which, although very popular with the news media, no doubt added fuel to the fires of disagreement and disbelief which followed the initial publications of the transfer results. Rosenblatt (1970) has suggested the term "behavioral induction" which,

although not as stirring as "memory transfer", probably is a more reasonable label for the phenomenon.

Memory transfer experiments have their origin in the research McConnell and his co-workers were doing with planaria (flat worms) in the late fifties and early sixties. Their most sensational result was the report in 1962 (McConnell 1962, 1964) that worms which were fed fragments of conditioned worms acquired the conditioned response more rapidly than other worms which ingested fragments of naive worms. These results apparently were the impetus for experiments involving rodents. In 1965, four original and independent descriptions of memory transfer in rodents were published (Fjerdingstad et al. 1965; Reinis, 1965; Ungar and Ocequera-Navarro 1965; and Babich et al. 1965). In three of these studies (Fjerdingstad, Reinis, and Babich) the working hypothesis was that RNA might somehow directly record the experience of the animal, and that an extract containing RNA might therefore transfer the learned behavior to a recipient. On the other hand, Ungar assumed that the molecule responsible for the transfer was a polypeptide. Rosenblatt (1970) lists several reasons for believing that the molecules responsible for transfer are polypeptides. Some of the reasons are: a) the greater facility with which peptides can get through the blood-brain barrier (Ungar 1970a), b) evidence of susceptibility of the extracts to proteolytic enzymes but not to RNase and, c) the finding of low molecular weights for the active factor (about 5000). It has been suggested (Guttman et al. 1972) that the results obtained with RNA extracts were actually mediated by peptide impurities for which RNA acted as a carrier. Guttman goes on to point out that "several

investigators now isolate these specific, behaviorally active peptides in the form of peptide-RNA conjugates (by perserving their artificial binding to RNA during an early isolation step), and then complete separation of the peptides from RNA by dialysis at low pH."

An early experiment which addressed itself to the specificity of the transferred response was due to Jacobson et al. (1965). Two groups of rats were trained in a Skinner box to approach a food cup when a discriminative stimulus was presented. One group was trained to respond to a click, the other to a blinking light. A brain extract was prepared and injected intraperitoneally into untrained rats. The two untrained groups then manifested a significant tendency (as compared with one another) to react differently to the two stimuli. That is, the group receiving extract from donors which had been trained to respond to the click responded to the click significantly more than they did to the blinking light, and those receiving extract from donors trained to respond to the blinking light responded to the blinking light significantly more often than they did to the click.

Similar results were obtained by Ungar (1967) when he habituated rats to either a loud sound or an air blast. The stimulus was repeated until the donor animals failed to startle in at least 90% of the stimulus presentations. Recipient animals were tested for their responses to both loud sounds and air blasts. The transfer of habituation was shown to be specific to donor training.

It is well known that interspecies transfer is possible. (Ungar 1968b, rats to mice, Babich et al. 1965b, hamsters to rats). Guttman et al. (1972) injected goldfish with synthetic scotophobin. Both intra-

peritoneal and intracranial injections were effective in passively transferring dark avoidance to the goldfish. Scores for scotophobin injected goldfish were significantly different from pretrial scores (P .001 Wilcoson test), whereas scores for placebo injected animals were not.

An attempt has been made to transfer instinctive behavior (Reinis and Mobbs 1970). About 30% of rats consistently kill mice, the others never kill them. Brain homogenates were prepared from both "killer" and "non-killer" rats. "Killer" extract was injected into "non-killer" rats and "non-killer" extract was injected into "killer" rats. In neither case was instinctive behavior altered. Under conditions similar to successful transfer of learned behavior transfer of instinctive behavior was not achieved.

If we can rule out transfer of non-learned behavior, the question then is, what types of learned behavior can be transferred? Provided the transferred response is part of the recipeint animal's innate ability, successful transfer has been demonstrated for the following learning situations: habituation, dark-light discrimination, spatial discrimination, conditioned reflexes, conditioned avoidance, and passive avoidance (Ungar 1970b). There is, however, an important additional qualification which must be made. It seems probable that only recently acquired behavioral traits can be transferred. Ungar (1970b) reports that prolonging the training period decreased the probability of transfer, and Adam and Faistz (1967) have reported that if the donors are over-conditioned, no significant transfer effect can be observed. Reinis and Mobbs (1970) report that a long delay between training and sacrifice was sufficient for elimi-

nating the transfer effect. Mice were fully trained and then left 15 days without further training. After 15 days they were tested and then killed. It was found that although the donors showed almost complete retention 15 days after training, their brain extracts were ineffective in causing transfer. These authors concluded that "memory transfer is a phenomenon connected with the process of training, not with the ready memory trace."

To summarize, it appears that the memory transfer phenomenon involves only recently learned behavior based on the recipients' innate responses.

APPENDIX B

STATISTICAL TESTS*

The data presented in the text of this dissertation can be put in the following form:

	Treatment 1	Treatment 2	Totals
Effect	a	c	a+c
No effect	b	d	b+d
Totals	a+b	c+d	N

where N = a + b + c + d. What is desired is a method for determining if there is a true difference between treatment 1 and treatment 2 with respect to the effect under consideration. The analysis is accomplished by hypothesizing that there is no true difference between treatment 1 and treatment 2 (the null hypothesis) and then calculating the probability that the hypothesis is true based on the observed differences between the two treatments. If this probability is sufficiently low, then the null hypothesis is rejected, and it is concluded that the difference between the two treatments is true. The probability that the null hypothesis is true is referred to as the level of significance.

The χ^2 Test

The chi-square test involves taking the difference between each

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*Much of Appendix B is taken from Croxton (1959) and Goldstein (1964).

observed frequency (f) and each corresponding expected, or computed, frequency (f_c), squaring this difference, dividing by the expected frequency and summing the quotients. Symbolically this is

$$\chi^2 = \Sigma \frac{(f - f_c)^2}{f_c} .$$

After the numerical value of χ^2 has been ascertained, the last step consists of determining the probability of its occurrence by chance.

An alternative procedure is available for computing χ^2 for 2 x 2 tables. This procedure allows χ^2 to be computed directly from the data in the table without the necessity of calculating the expected frequency. The equation for χ^2 then becomes

$$\chi^2 = \frac{(ad - bbc)N}{(a+b)(c+d)(a+c)(b+d)} .$$

From a table of χ^2 we may determine the probability of the chance occurrence of χ^2 greater than or equal to the calculated value of χ^2 for one degree of freedom. The reason why the number of degrees of freedom is equal to one is that, for a 2 x 2 table with marginal totals set, a frequency may be entered freely in but one of the four cells. Once one frequency has been entered the other three are determined by the marginal totals.

It should be noted that the χ^2 test is a two-tail test. That is, the χ^2 test provides the probability of obtaining a discrepancy equal to

or greater than that observed in either direction. This is so because the $f - f_c$ values are squared.

χ^2 Test With Yates Correction

The χ^2 distribution is continuous, while the actual distribution of the data is discontinuous. This does not introduce any appreciable error as long as N is large ($N > 100$). However, for small N a correction must be made to account for the discontinuity. This can be done by using Yates correction, which consists basically of decreasing each $|f - f_c|$ by one-half. Expressed in terms of the data from the table, the expression for χ^2 with Yates correction is

$$\chi^2_Y = \frac{(|ad - bc| - \frac{1}{2}N)^2 N}{(a+b)(c+d)(a+c)(b+d)} .$$

The Exact Method

Although Yates correction to the χ^2 distribution makes an adjustment for discontinuity in the data, it does not allow for skewness. Both discontinuity and skewness are taken into consideration by the exact method. The rationale of the method is simply that for given marginal totals the entries in the boxes may be arranged in a number of different ways. All possible 2 x 2 tables with fixed marginal totals can be written and the probability of each such table, if the null hypothesis was true, can be calculated. The probability of obtaining the particular table in hand, by chance, is thus obtained. This probability is given by

$$P = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{N!a!b!c!d!} .$$

The desired probability is for obtaining the particular table or any other table which shows an equal or greater divergence between the two treatments than the particular table in hand. Thus, the probabilities for the table in hand and all tables either equally extreme or more extreme must be summed.

A one-tail test can be made provided the hypothesis under which the experiment is conducted allowed a prediction of the direction of the divergence in the data. In this case only the probabilities of the combinations of frequencies which show a divergence in the predicted direction equal to or greater than that shown by the sample need be considered.

APPENDIX C

SAMPLE DATA AND CALCULATIONS

For experiments involving groups 1 through 8, each litter was weighed, injected, and tested on a schedule stated in Chapter V. Each litter is identified by its date of birth and, in those cases where more than one litter was born on a given day, a litter number. Tail markings with a waterproof dye distinguished recipients from their litter-mate controls. After weighing and injecting the litters, they were exposed to the bell at ages 21 and 24 days. Their responses to the bells were recorded as either no convulvion (0), wild running fit (1), clonic convulsion (2), tonic convulsion (3), or death from respiratory failure (4).

Data For "October 15 Litter 1" From Experimental Group 5

4 November - Weighed "Oct. 15 litter 1"

9 mice = 73 gm ave. = 8.1 gm

Injected "Oct. 15 litter 1" 11:15

5 November - Tested "Oct. 15 litter 1" 12:30

No convulsions in either recipient or control groups.

8 November - Tested "Oct. 15 litter 1" 12:15

Recipients	Controls
M 1	M 1
F 4	M 1
F 1	F 1
M 1	F 1
	M 1

Data and Calculation For Experimental Group 5

Experimental group 5 was composed of six litters of mice, one of which was "Oct. 15 litter 1." Data were recorded for these litters in the manner indicated in the previous example. The responses of group 5 mice to the test bell at 24 days of age are given in Table 4. M and F indicate the sex of the experimental animal.

In experimental group 5 the fraction of recipients having at least tonic convulsion is seven out of 21 whereas it is one out of 21 for the litter-mate controls. These data can be put in the form of a contingency table for statistical analysis:

	Recipients	Controls	Totals
At least tonic convulsion	7	1	8
Less than tonic convulsion	14	20	34
Totals	21	21	42

The probability that the recipients and controls are part of a common populations and that the observed differences between them are due to chance can be calculated by a one-tail, exact method calculation:

$$P = P\left(\frac{7 \mid 1}{14 \mid 20}\right) + P\left(\frac{8 \mid 0}{13 \mid 21}\right)$$

$$P = \frac{21!\ 21!\ 8!\ 34!}{42!\ 7!\ 1!\ 14!\ 20!} + \frac{21!\ 21!\ 8!\ 34!}{42!\ 8!\ 0!\ 13!\ 21!}$$

$$P = 0.021 + 0.0015 = 0.0225.$$

Thus, the injection of primed brain extract is deemed to be effective in increasing susceptibility to audiogenic seizure at the 2.25% level of significance.

Table 4. Responses of Experimental Group 5

Litter	Recipients		Controls		Average weight per mouse
Oct. 14	M	4	F	1	9.1 gm
litter 1	M	1	M	1	
	F	1	M	1	
			M	1	
Oct. 14	F	4	M	1	8.1 gm
litter 2	F	4	M	1	
	M	1	M	1	
	M	1	M	1	
	M	1			
Oct. 14	F	1	F	4	8.2 gm
litter 5	M	4	M	1	
	M	4	M	1	
	M	1	F	1	
Oct. 15	M	1	M	1	8.1 gm
litter 1	F	4	M	1	
	F	1	F	1	
	M	1	F	1	
			M	1	
Oct. 15	F*	1	M	1	10.2 gm
litter 2	F	1	F	1	
Oct. 16	M	1	F	1	8.4 gm
litter 2	M	4	M	1	
	F	1			

* Wild running fit in response to exposure to first test bell.

Calculation For Experimental Groups 5 and 6

On the assumption that the control injectants (physiological saline and extract from non-primed mice) do not differ from one another with respect to their ability to effect audiogenic seizure susceptibility, experimental groups 5 and 6 were combined, and the data were put in the form of a contingency table for statistical analysis:

	Recipients	Controls	Totals
At least tonic convulsion	15	6	21
Less than tonic convulsion	24	37	61
Totals	39	43	82

The level of statistical significance was determined using a χ^2 test with Yates correction:

$$\chi^2 = \frac{[(15)(37) - (6)(24) - \frac{1}{2}(82)]^2 \, 82}{(39)(43)(61)(21)} = 5.2.$$

With one degree of freedom, $\chi^2 = 5.2$ yields $0.02 < P < 0.025$.

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VITA

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